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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS, PHASE III. EFFECTS O--ETC(U)
JAN 80 H V ELLIS, J H HAGENSEN, J R HODGSON DAMD17-74-C-4073

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MIDWEST RESEARCH INSTITUTE

REPORT

MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS
PHASE III: EFFECTS OF LIFE-TIME EXPOSURE
PART III: NITROCELLULOSE

PROGRESS REPORT NO. 9

January [redacted] 1980

Contract No. DAMD-17-74-C-4073
MRI Project No. 3900-B

For

Contract Officer's Technical Representative: Dr. Jack C. Dacre
Environmental Protection Research Division
U.S. Army Medical Bioegineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

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Animal Experimentation: Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1974) prepared by the Institute of Laboratory Animal Resources, National Research Council· the regulations and standards prepared by the Department of Agriculture: and Public Law 91-570, "Laboratory Animal Welfare Act," 1970.

Disclaimer: The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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Risk Assessment	Water Quality Criterion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effects of feeding nitrocellulose (NC) at levels up to 10% in the diet were studied in dogs, rats and mice. A cotton control (fed 10% cotton linters) was included with each species. Ancillary studies included cytogenetic analysis and a three-generation reproduction study in rats.

In all species, the NC appeared to be inert dietary bulk, since there was a dose-related increase in feed consumption. No other effects were seen in dogs. In rats and mice, those fed 10% fiber (NC or linters) had decreased

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weight gain. This was not sufficient to be adverse except in some rats with high nutrient demand from pregnancy and lactation. Some mice fed 10% fiber died early of intestinal impaction from the fiber: Some mice died about month 9 of feeding due to an unexplained mechanism. All effects, except the Month 9 deaths in mice, were at least as severe in animals fed 10% cotton linters as in those fed 10% NC, and are ascribed to the effects of the fiber itself.

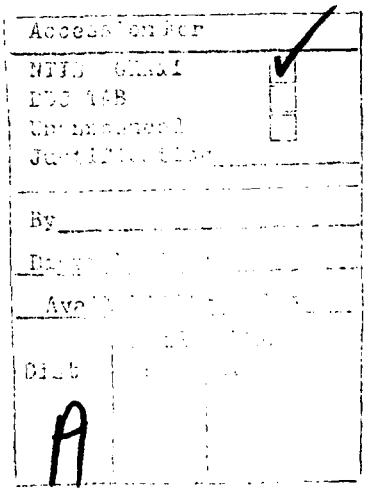
Because of this lack of toxicity from large doses of ingested NC, we conclude that water quality criteria should be based on physical factors, such as clarity and total suspended solids.

FOREWORD

The U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL), Fort Detrick, Frederick, Maryland, has been conducting a research program since 1973 for the purpose of developing the scientific data base from which water quality criteria for compounds unique to the munitions industry could be determined. A water quality criterion (as defined by the amended Clean Water Act, 1977) is a qualitative or quantitative estimate of the concentration of a pollutant in ambient waters that, when not exceeded, will ensure a water quality sufficient to protect a specified water use. The criterion is a scientific entity based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility. Currently, a water quality criterion consists of two separate numerical limits, one for the protection of human health and the other for the protection of aquatic organisms. These numbers, when translated by the appropriate regulatory agency, can be the basis of enforceable discharge or effluent limitations in a point source discharge permit issued under the Clean Water Act.

Since a water quality criterion is to protect designated water uses, a diverse, multidisciplined research program was developed by USAMBRDL that includes "effects" studies on laboratory and domestic animals, wildlife species, aquatic organisms, plants, and economically important crops. In addition, extensive chemical and biological fate and persistence tests are conducted to provide information on the behavior of a pollutant in the aqueous environment. These kinds of data are especially useful for making site-specific translation of criteria into enforceable discharge limits.

This report represents a portion of the mammalian toxicology data base being developed by USAMBRDL on materials related to the use and disposal of nitrocellulose.



PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under U.S. Department of the Army Contract No. DAMD-17-74-C-4073, MRI Project No. 3900-B, "Munition Compounds Mammalian Toxicity Study." The work was supported by the U.S. Army Medical Research and Development Command, Department of the Army. Dr. Jack C. Dacre, Environmental Protection Research Division, USAMBRDL, was the contract officer's technical representative for the project.

This work was conducted in the Biological Sciences Division under the direction of Dr. William B. House, between March 1, 1975 and March 31, 1978, and Dr. Harold M. Hubbard, between April 1 and September 30, 1978. The experimental work was directed by Dr. Cheng-Chun Lee, Deputy Director, with the assistance of Dr. Harry V. Ellis, III, Senior Pharmacologist. Mr. Jack H. Hagensen, Supervisor, supervised the animal experimentation with the technical assistance of Karen J. Smith, E. Renee Walton, Darrel L. Lavish, Pam J. Saunders, Linda J. Ryhal and J. Christopher Unger. Dr. John R. Hodgson, Head, Biochemical and Developmental Pharmacology, supervised the studies on cytogenesis and mutagenesis, with technical assistance of Daniel L. VanGoethem, Mary A. Kowalski and Maxine Hainje. Mr. Jan L. Minor, Assistant Toxicologist, supervised reproduction studies and the computer program and analysis of experimental data, with technical assistance of Timothy M. Unger. Dr. C. B. Hong, Senior Veterinary Pathologist, supervised the necropsy and the histology preparation and with Dr. L. D. Thornberg, Consulting Pathologist, performed the microscopic examination, with Dr. L. D. Thornberg, Consulting Pathologist, performed the microscopic examination, with technial assistance of Ellen R. Ellis, Kerry L. Crabb, Janet Kliethermes, Ernesto A. Castillo and Judith Shifrin. Miss Judith D. Girvin (ASCP certified M.T.), Laboratory Supervisor, supervised the hematology and clinical laboatory tests, with the technical assistance of Ilonna S. Elwood, Duane R. Smith and Bhanu S. Gosalia. Dr. Betty L. Herndon, Associate Pharmacologist, prepared the water quality criterion.

Approved for:

MIDWEST RESEARCH INSTITUTE

Florence I. Metz, Acting Director
Pharmacology/Toxicology Department

August 1979

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EXECUTIVE SUMMARY

The effects of nitrocellulose (NC) after feeding for up to 2 years were studied in dogs, rats and mice. The concentrations of NC in feed (on a dry basis) used in all studies were 0 (control), 1%, 3% and 10%. To determine whether observed effects were due to the NC itself or merely due to the fibrous nature of the NC, a "cotton control" group fed 10% cotton linters, the raw material for NC, was included with each species. Ancillary studies included a three-generation reproduction study in rats and cytogenetics assay in dogs and rats.

Most observed effects were seen in both NC-fed and cotton linter-fed animals, so they were called "fiber effects." In general, NC acted as if it were inert dietary bulk.

The only effect observed in dogs was a somewhat higher feed consumption in the fiber-fed dogs. The increases were proportional to fiber content. Rats and mice had similar effects. However, the rodents fed 10% fiber (NC or linters) had a much higher apparent feed consumption than the other groups because they removed some of the feed mixture from the feeders and separated the feed itself from the fiber. In some cases they even nested in the fiber. Rats fed 10% fiber had lower body weights than controls, due to decreased fat, and lived somewhat longer.

Additional effects were seen only in mice. Some could not eliminate the fibers through their gastro-intestinal tracts. These mice died of intestinal impaction in the first weeks of the study. A number of mice fed 10% fiber had transient hyperemia of the extremities, ascribed to irritation from the fibers scattered about their cages. In month 9 there was a cluster of deaths of unknown cause(s) in the 10% fiber mice. There were three times as many deaths in the 10% NC-fed mice as in the cotton-fed mice. This is the only case in which the NC had a greater adverse effect than the fibers.

Cytogenetics studies found no adverse effects in dogs and rats fed NC for 24 months.

In the three generation reproduction study, the rats (sires, dams and pups) in the 10% fiber groups were generally lighter than the others. During the first two generations the 10% fiber pups had decreased survival to lactation, because the dam could not get enough nutrition from the feed-fiber mixture. The third-generation dams did adapt, and had normal survival rates and pup weights.

I. INTRODUCTION

Under Contract No. DAMD-17-74-C-4073, entitled "Munition Compounds Mammalian Toxicity Study," we have performed a variety of studies, divided into three phases. Phase I, Effects of Acute Exposure, includes acute oral toxicity, primary skin and eye irritation, dermal sensitization, and disposition and metabolism studies. Results were reported in Progress Report No. 1.[1] Results on additional compounds plus in vitro mutagenic (Ames test) studies were submitted as Report No. 6.[2] Phase II, Effects of Multiple Exposure, includes subacute and subchronic toxicity, reversibility, immunologic response, chemical-biological interaction, mutagenicity, and disposition and metabolism studies. Results were presented in a series of reports on the compounds tested, trinitroglycerin (TNG),[3] 2,4-dinitrotoluene (2,4-DNT),[4] 2,6-dinitrotoluene,[5] and nitrocellulose (NC).[6] Phase III, Effects of Life-Time Exposure, includes chronic toxicity, reversibility, reproductive, cytogenetic, and metabolism studies. The results on 2,4-DNT and TNG were submitted as Report Nos. 7 and 8.[7,8] This report contains the results of studies on NC.

II. MATERIALS AND METHODS

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II. MATERIALS AND METHODS

Materials and methods employed in these studies are described below.

A. Animals

1. Sources

Young, healthy beagles were bought from Hazleton Research Animals (Cumberland, Virginia). Young healthy CD® rats and CD-1® mice were bought from Charles River Breeding Laboratory (Wilmington, Massachusetts). All animals were maturing. They were conditioned in our animal quarters for at least 2 weeks.

2. Housing and Animal Husbandry

Dogs were kept in dog pens with outside runs. Up to 12 dogs shared 60 sq ft of heated inside space and 120 sq ft of outside space. Water was available continuously. Dogs were fed as described below under feed measurement. Runs were cleaned daily.

Rats and mice were kept in plastic cages with hardwood chip bedding, metal lids and filter tops. Bedding was steam-sterilized before use and changed at least weekly. Cages, tops and water bottles were steam-sterilized before use and changed weekly. Feed and water were available at all times. Usually two male rats, three female rats, four male mice, or four female mice were housed in each cage and differentiated by earpunches. Some groups (especially male mice) were subdivided to prevent fighting. The rodent quarters are fully air conditioned, with 10 air changes per hour, maintained at 75 $\pm$ 5°F and 50 $\pm$ 10% relative humidity. The room air is passed through filters to remove 99.9% of all particles larger than 0.3 μ. Lighting is controlled by a timer providing 12 hr on and 12 hr off.

All animals were observed daily for toxic signs and behavioral changes and were provided medical treatment as necessary for nontest injuries under the supervision of our veterinary pathologists. The typical case was injuries due to fighting, which may be treated by isolation, cleaning the wounds, and antibiotic therapy, systemic and local.

B. Basic Protocol

1. Dose Levels and Treatment

We used two control groups and three treatment groups, with the compound given in the feed to all three species. The treatment groups received 1% (low), 3% (middle), or 10% (high) NC in feed, calculated on a dry basis. The normal control group received plain feed; the second control group, called the "cotton control," received a diet containing 10% cotton linters (cellulose linters, Military Specification MIL-C-20330, Hercules, Inc., Wilmington, Delaware). This material is nitrated to form NC. This second control group served to determine if the passage of a non-nutritive bulk through the gastrointestinal tract will cause any effects.

Diets for the dogs were prepared daily. Damp NC was mixed with appropriate amounts of Champion Dog Food (kennel formula, Tri-Foods, Inc., Concordia, Missouri) and water to produce a 10% NC mixture using a Univex Model 1222 food mixer with a wire whip beater. Aliquots of this diet were mixed with additional dog food to produce the 3 and 1% diets. Linters were loosened from the bale with the wire whip, then mixed with appropriate amounts of the dog food and water to produce the "cotton control" diet (10% linters). The dog food was moistened to produce the normal control diet. Extra amounts of these mixtures were prepared on Friday and refrigerated for feeding over the weekend.

Diets for the rodents were prepared weekly. The methods were similar to the dog feed, except that powdered rodent chow (Wayne Lab-blox® Allied Mills, Inc., Chicago) was used and the mixtures prepared in a rotating box on a modified cement mixer. Diets were refrigerated to prevent spoilage of the wet mixture before it was given to the animals.

2. Number of Animals and Identification

Each group consisted of equal numbers of males and females. The beginning number of dogs was six of each sex per group, of rats 32 and of mice 58. An additional 20 female rats in each dosage group were included for the three-generation study. A few extra rodents were added to replace early losses. A separate group of rats, eight of either sex per dose group, was begun 6 months later and used for the 1 year necropsy.

Each animal is assigned a three- to five-digit number. The first two digits indicate the dosage groups for NC, i.e., 50, 51, 52, 53 and 54 for the cotton control, control, low, middle, and high dose groups, resepectively. The last one or two digits (dogs) or three digits (rodents) are the animal numbers within each species. Eight rats of each sex in each dose group have prefixes of 40 through 44; these were originally intended for a subchronic study, but were continued as part of the chronic study.

3. Schedule

a. Dogs: All the dogs were bled from their jugular veins for hematology and clinical chemistry tests before dosing and at the end of 3, 9, 12, 18 and 24 months during dosing. The dogs were weighed weekly; their feed consumptions were measured 1 week each month. After 12 months dosing, one male and one female dog from each dosage group were killed for necropsy. The treatment of a second pair was discontinued for 4 weeks. These dogs were used on a recovery study and killed for necropsy at the end of 13 months. After 24 months' feeding, two males and two females from each dosage group were killed for necropsy. The remaining dogs were used on a recovery study for 4 weeks and terminated at the end of 25 months.

b. Rats: Four males and four females from each dosage group were bled for hematology by cutting off their tail tips before dosing and at the end of 6, 12, 18 and 24 months during dosing. As much as possible, the same rats were used at each bleeding. If a bled rat died or his tail became too short, another rat was substituted. Rats were weighed weekly for the first 6 months; after weight gain leveled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then during the last week of each month. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for clinical chemistry and killed for necropsy. A second group of four male and four female rats from each dosage group was started on a recovery study without treatment for 4 weeks. These rats were terminated at 13 months. The later-starting rats were used for these two (12 and 13 month) studies. After 24 months dosing, a similar recovery study was started and the remaining surviving rats were killed for necropsy, with eight from each group bled for clinical chemistry. The recovery rats were terminated at the 25th month.

c. Mice: Mice were weighed weekly for the first 5 months; after their weight gain leveled off, they were weighed biweekly. Feed consumption was measured during the first 4 weeks and then for 1 week each month thereafter. After 12 months dosing, four males and four females from each dosage group were bled from their aortas for hematology and killed for necropsy. A second group of four male and four female mice from each dosage group was started on a recovery study. After 24 months dosing, a similar recovery study was started and the other surviving mice killed for necropsy, with eight mice from each dosage group bled for hematology. The recovery mice were terminated at the 13th or 25th month, respectively.

C. Test Compound

1. Preparation

We used military production NC from Radford Army Ammunition Plant (Radford, VA). Originally, the NC was dipped from the poacher pits (the last purification step, in which the NC is washed with several charges of boiling water) and shipped to MRI in 55 gal. drums. As needed, NC was removed from the drums and dewatered with a Buchner funnel attached to a water aspirator vacuum pump. Later batches were taken from the first press (the first step downstream from the poacher pits, where drying begins) and did not require dewatering before mixing. NC was always kept damp, except for small samples dried to determine moisture content so that proper amounts of wet NC would be used to provide the desired concentrations of NC in the feed mixtures. Unused NC, feed mixtures, feces, and bedding were collected in closed metal containers and burned in an open pit.

2. Nitrocellulose Studies

The "fines" of the NC were characterized physically and chemically. Results have been reported.[18]

3. Feed Assays

The procedure involves a wet titration using chromous chloride as a reducing agent for the nitro groups.[19] Radford Army Ammunition Plant reports the Nitrocellulose Poacher Pit Fines to contain 13.08% nitrogen, we observed 13.0 $\pm$ 0.1% nitrogen. The presence of rat feed caused no interference with this assay method.

4. Mass-Balance Metabolism Study of Nitrocellulose

A dog was fed 90 g of wet nitrocellulose (27.9 g on a dry basis). After 24 hr the feces were collected and analyzed. The feces (50.3 g wet weight) contained 8.8 g of nitrocellulose. After 48 hr an additional 16.2 g feces contained 0.7 g nitrocellulose. After 72 hr an additional 8.3 g feces contained 0.0 g nitrocellulose. After 96 hr an additional 23.4 g feces contained 0.0 g nitrocellulose.

Over a 4-day period 9.5 g of NC were recovered. This represents a recovery of 34%.

a. Recovery study: The accuracy of our handling and assay procedure was determined. In the above work the percent nitrocellulose per weight of feces after 24 and 48 hr was 17.4 and 4.3%. Accordingly feces samples were spiked at similar levels. Thus, 2.1 g feces was spiked with 62.6 mg nitrocellulose (3.0% spike) and 2.1 g feces was spiked with

291.3 mg nitrocellulose (13.9% spike). After standing 24 hr at room temperature the samples and a blank were freeze dried. The samples were then transferred to 250 ml bottles, 100 ml dimethyl formamide added, followed by homogenization for 2 min using a Polytron homogenizer. After centrifugation aliquots were taken for assay by the method of Walter Selig.[19] From the 13.9% spike level a recovery of 99% was observed. From the 3.0% spike level a recovery of 92% was observed. The blank sample required 0.0 ml titrant.

b. Comments on calculations: The assay procedure required reduction of the nitro groups with chromous chloride, followed by titration of the excess chromous chloride with ferrous ammonium sulfate to a colorimetric endpoint.

Our nitrocellulose contains 13.08% nitrogen. This corresponds to 92.5% nitration (14.14% N is maximum). The formula for nitrocellulose is $C_6H_7O_2(ONO_2)_x(OH)_{3-x}$, where x is the degree of nitration. Our 13.08% nitrogen content corresponds to a 2.775 degree of nitration (3 x 0.925). Therefore, the average molecular weight of the nitrocellulose monomer is 286.8 and the molecular weight of the $(ONO_2)_x$ group is 172.05. Thus, 1 mg of this nitrocellulose corresponds to 0.6 mg ONO_2. The concentration of NC in a sample can now be calculated using the following relationships.

$$\text{mg of NC present} = y \text{ mg } ONO_2 \times \frac{1 \text{ mg NC}}{0.6 \text{ mg } ONO_2} \times \text{dilution factors}$$

where $y = \frac{(A-S)\ D}{A-B}$

and A = ml $Fe_2(SO_4)_4(NH_4)_2$ to titrate blank

B = ml $Fe_2(SO_4)_4(NH_4)_2$ to titrate standard ONO_2 solution

S = ml $Fe_2(SO_4)_4(NH_4)_2$ to titrate sample

D = mg ONO_2 present in standard ONO_2 solution.

D. Procedures

1. Observation

All animals were observed daily for toxic signs and changes in behavior and general health.

2. Body Weights

Body weights were taken as mentioned above. Dogs were weighed to 0.1 kg, rodents to 1 g.

3. Measurement of Feed Consumption

The feed consumption of the dogs was measured by placing them in a metabolism cage, giving them a measured amount of feed, waiting 0.5 hr, then returning them to their pen and estimating the remaining amount of feed by volume. This value was converted to weight by a factor determined by averaging the weight of 20 replicates of volume measurements. Feed consumption of the rodents was determined by weighing the feed and container placed in the cage and that remaining 1 week later.

4. Unscheduled Deaths

If an animal appeared moribund, he was killed and necropsied as described below. If an animal was found dead, he was necropsied as thoroughly as possible, but no blood samples or organ weights were taken. If an animal received a serious injury or lesion, causing pain and suffering (such as an ulcerated tumor), he was killed and necropsied as if moribund.

E. Hematology and Clinical Chemistry

1. Hematology

The hematology battery included erythrocyte, reticulocyte, leucocyte and platelet counts, hematocrit, hemoglobin, erythrocyte indices, methemoglobin, Heinz bodies and (for dogs) clotting time. Details of methodology are summarized in Appendix I.

2. Clinical Chemistry

The clinical chemistry battery included fasting blood glucose, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase, and blood urea nitrogen. Details of methodology appear in Appendix I.

3. Immunoglobin E

Immunoglobin E (IgE), the allergic or hypersensitive antibody, was associated with anaphylactic reactions in humans.[9] Serum concentrations of IgE were determined in all clinical chemistry samples, using the immunodiffusion technique of Mancini.[10]

4. Statistics

Data were analyzed using Dunnett's multiple comparison procedure following an analysis of variance, as described in Appendix I.

F. Necropsies

1. Killing and Gross Examination

Rodents were killed with ether and dogs with an overdose of sodium pentobarbital. The necropsy was performed as soon after death as possible. Gross abnormalities in all tissues are observed and recorded.

2. Organ Weights

The brain, heart, liver, kidneys, spleen, gonads, and (dogs only) adrenals, thyroids and pituitary were trimmed free from surrounding tissues and weighed. The absolute weights and organ weight to body weight and/or

brain weight ratios were analyzed statistically. Abnormal growths were measured and, if practical, trimmed and weighed.

3. Histopathology

Tissues routinely taken for histopathologic examination are listed in Table 1. In addition, all tissues with gross abnormalities were taken. Processing is detailed in Appendix I.

G. Recovery Studies

Recovery studies were performed after each scheduled necropsy (12 and 24 months). The compound treatment of one male and one female dog or four rodents of each sex was discontinued. They were given the control treatment (feed without compound) for 28 days. During this period, their weight and feed consumption were determined weekly. At termination, blood samples were taken from the jugular vein of dogs or the aorta of rodents for hematology and (except mice) clinical chemistry. The animals were then killed and necropsied. Detailed procedures are as given above.

H. Three-Generation Reproduction Study

1. Study Design

The study design is illustrated in Figure 1. The initial groups of rats used as the parental generation (F_0) were started at the same time as the chronic toxicity study. Rats of each group, parents and offspring of each generation, received the same control or NC-containing diets as in the chronic study. For the F_0 generation, 10 males and 20 females from each dosage group were mated after receiving the test diets for 6 months. Each male was housed with two females from the same dosage group for 14 days. Offspring from the matings (F_{1a}, first litters) were discarded at weaning. The F_0 rats were again mated. Twenty to 24 offspring of each sex from this mating (F_{1b}, second litters) were radonmly selected (with approximately equal numbers of pups from the various litters) from each dosage group at weaning. The F_0 females and surplus pups were discarded; the F_0 males were retained in the chronic study. Each F_{1b} male was mated with a female within the same dosage group for 14 days at 3 months of age. The F_{2a} generation was discarded at weaning and the F_{1b} rats were terminated at weaning of the F_{2b} pups. The F_{2b} rats were then selected and mated at 3 months of age according to the same procedure used for F_{1b}. The study was terminated upon weaning of the F_{3b} rats.

2. Evaluation

At birth, all offspring were examined for gross physical abnormalities and the number of live and dead pups of each litter were recorded. Survival and body weights were recorded at 0, 4 and 21 days.

Reproductive performance for each parental generation was quantified by: the mating ratio (the number of copulations to the number of malefemale pairings), and fertility ratios for each sex (the number of males or females with offspring to the number of that sex mated). Reproductive performance for each litter was quantified by: the litter size, the liveborn index (the percentage of the total number of pups liveborn), the weight of liveborn pups at birth, the viability index (the percentage of liveborn pups surviving to 4 days), the lactation index (the percentage of the young alive at day 4 surviving to weaning), the weight at weaning, and the sex ratio (the number of males to the total number of offspring). Details of procedures appear in Appendix II.

The general health of the parental generation was quantified by the weight at first mating.

I. Mutagenesis Studies

To assess the mutagenic potential of NC, we performed cytogenetic analysis of tissue cultures from rats from the chronic toxicity study.

1. Preparation of Cell Cultures

At the end of 1 year, blood samples were aseptically drawn from both cotton control and treated rats. Blood was obtained from the tail vein of the rats. The lymphocytes were cultured by the method of Moorhead et al.[11] Bone marrow cells replaced peripheral blood lymphocytes as a source of mitotic chromosomes in the 2year study. The use of bone marrow cells rather than peripheral blood lymphocytes has several advantages. Chromosomes will be obtained not only from lymphoidal cells but also from cells of myeloid, erythroid, and reticuloendotheloid origin. Another advantage of bone marrow cells is that the culture time is reduced from 72 hr needed in lymphocyte cultures to 24 hr and no mitogenic agent is required to obtain metaphase chromosomes. Femur bone marrow was removed at necropsy and processed by the method of Eggen;[12] bone marrow cultures were maintained in nutrient mixture F12 (HAM). Kidney tissue samples were removed at necropsy, cultured by the trypsinization method of Fernandes,[13] and maintained in Eagle's medium as modified by Dulbecco and Vogt.[14]

2. Chromosome Analysis

Actively dividing kidney cultures, bone marrow cells, and phytohemaglutin-stimulated lymphocytes were arrested in metaphase by short-term colchicine treatment. The cells were removed from the culture flasks, swollen in hypotonic solution, and processed for spreading on glass slides by the method of Moorhead and Newell.[15] Slides were stained with Giemsa and scanned under low power optics. The slides showing minimum scattering of cells were selected for analysis under oil immersion optics. Cell ploidy was estimated by examination of 200 cells. Chromosomes were counted and morphological aberrations were examined from photographic negatives of up to 50 metaphase cells.

TABLE 1

ORGANS ROUTINELY TAKEN AT NECROPSY

Thyroid and parathyroids	Caecum
Pituitary	Colon
Adrenals	Urinary bladder
Lungs	Ureter[a/]
Liver and gallbladder	Diaphragm[a/]
Spleen	Skeletal muscle
Heart	Esophagus
Salivary glands	Tonsils[a/]
Pancreas	Mesenteric lymph node
Thymus	Tongue[a/]
Prescapular lymph node[a/]	Skin
Gonads	Mammary gland
Uterus or prostate and accessory organs	Brain
	Spinal cord[a/]
Stomach	Sciatic nerve[a/]
Duodenum	Eyes
Jejunum	Trachea
Ileum	Rib and bone marrow

a/ Not normally removed from rodents.

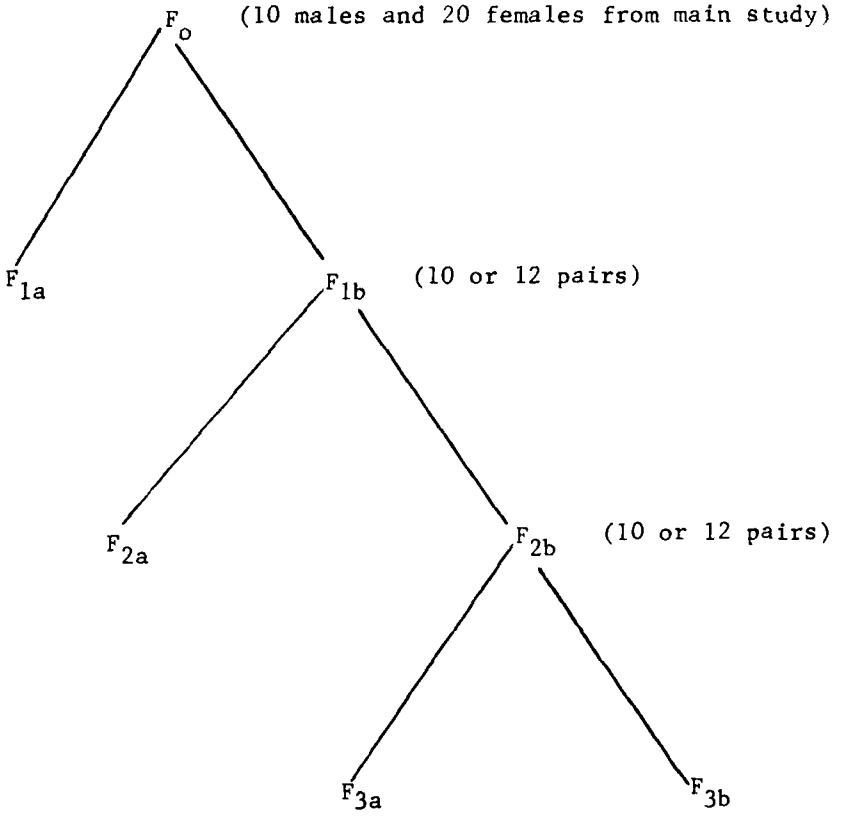

Figure 1 - Design of Three Generation Reproduction Study

III. DOG STUDIES

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III. DOG STUDIES

The following sections describe the results and interpretations of the dog studies.

A. Observations and Toxic Signs

No toxic signs were seen in any of the dogs at any time. However, a variety of non-treatment related problems occurred. For instance, in Month 8, cotton control male (10% linters) No. 50-15 developed an abcess in his jaw, which caused him to decrease feed consumption. The abcess was drained, and antibiotic therapy resulted in rapid healing.

Most other observed signs were injury due to fighting. Recovery usually occurred. During the cold of the first winter (about Months 10 and 11), there were a number of severe fights. The low dose (1% NC) male No. 52-21 and middle dose (3% NC) males Nos. 53-63 and 53-65 were kept isolated in metabolism cages for several weeks until their wounds healed. The high dose (10% NC) female No. 54-70 in the recovery study after 12 months feeding was found dead after the weekend of the third week of recovery, with many cuts and bites all over her body. Control male No. 51-1 was a repeated victim of bites. In Month 18, he was found bleeding again and died. Necropsy found punctures of the lung, liver and spleen.

The second winter (Months 22 and 23) was even more severe, with over a month of continuous snow cover. The coldest day was a Sunday with a high of -2°F. The next day, we found low dose male No. 52-17 lying in the outside run, suffering from wounds and hypothermia. He lost consciousness, then died. His terminal rectal temperature was 94.6°F; normal is near 100°F. The next day, cotton control female No. 50-16 was similarly victimized, but remained inside the building. She was less badly injured and recovered after the wounds were treated. After the weather improved, the fights continued. Middle dose male No. 53-31 had a hematoma of the left lower eyelid among other injuries, apparently without injury to the eye itself. Low dose male No. 52-25 injured his left hind leg on the metal bench in the run. High dose female No. 54-38 had a rectal prolapse in Month 24. These dogs fought more than dogs in other studies. The fighting occurred in all dosage groups.

B. Body Weight and Feed Consumption

The average body weights of dogs fed NC or linters are shown in Figure 2; data from dogs fed 1% or 3% NC are omitted for clarity; some data were missing. Weights of dogs varied, but there were no dose-related variations. Among males, the controls were usually the heaviest, and the middle-dose dogs the lightest. Among the females, the cotton controls and controls

were often slightly heavier than the other groups, but this was not consistent. There is an overall trend to increasing weight in all groups, reflecting both maturation and obesity. Notable were cotton control male No. 50-9, with a terminal weight of 20.8 kg and middle dose female No. 53-28, with a terminal weight of 15.2 kg. Both were quite obese.

Average feed consumptions are shown in Figure 3. Again, data for dogs fed 1% or 3% NC are omitted for clarity. Overall averages are given in Table 2. Dogs fed 10% NC or linters ate considerably more than the control dogs. Dogs fed lower doses ate only slightly more than controls. Considering the inherent variation in the measurement method (based on visual estimation of feed left in the bowl) and the inconsistency between the sexes, the difference of feed consumption between the 10% NC and 10% linters groups is not considered to be toxicologically important.

C. Laboratory Data

Baseline values (two assays on each dog) of hematology and clinical chemistry for male and female dogs are shown in Tables 3 and 4, respectively. The following tables show the values for these dogs after being fed linters or various doses of NC for 3 months (Tables 5 and 6), 9 months (Tables 7 and 8), 12 months (Tables 9 and 10), 18 months (Tables 11 and 12) and 24 months (Tables 13 and 14).

Before the start of the study, the only data significantly different from those related to the control dogs were some variations in the differential lymphocyte counts of the middle dose and cotton control males, a high mean corpuscular hemoglobin in the high dose males and a high count of nucleated erythrocytes in some of the high dose females. These variations are all small and within normal limits (see Appendix I).

During the study, results were similar with occasional small and inconsistent changes, within normal limits, of various parameters in various groups. No abnormalities were dose-related, and none persisted. Therefore, they are considered to be normal variations, not related to the NC feeding.

D. Pathology

1. Feeding for 12 Months

A few gross changes, not related to the NC feeding, were seen at necropsy. The control male (No. 51-51) had several circular gray to white spots on his lungs; he and the control female (No. 51-52) had roundworms. The low dose male (No. 52-59) and the high dose male (No. 54-67) had somewhat discolored livers.

There were no apparent effects of NC feeding on the organ weights (Table 15), although there were some variations among the individual dogs.

Histopathological examination (Table 16) found a variety of minimal to mild lesions in all the dogs and moderate thyroid hyperplasia in the control and high dose females (Nos. 51-52 and 54-68, respectively) and moderate thymus involution in the cotton control male (No. 50-55). These lesions are typical of those naturally occurring in dogs of this age.

Since there were no effects related to NC treatment, the slides from the low and middle dose dogs were not read, and the necropsy on the recovery dogs was omitted after discussion with the technical monitor.

2. Feeding for 24 Months

A variety of minor lesions were seen on gross examination. Pituitary cysts were noted in middle dose female No. 53-26 and cotton control female No. 50-12. There were lung lesions in control male No. 51-3, low dose male No. 52-21, middle dose males Nos. 53-25 and 53-27 and females Nos. 53-26 and 53-28, high dose male No. 54-35 and females Nos. 54-34 and 54-36 and cotton control males Nos. 50-9 and 50-11. Foreign bodies in the intestine included worms in control female No. 51-12 and a rubber glove in low dose female No. 52-18. Low dose male No. 52-21 had "cherry eyes." Middle dose male No. 53-25 had almost healed wounds on the right hind leg and an ectopic spleen.

The absolute and relative organ weights (Table 17) had some variations between individual dogs, but no differences between dogs in different treatment groups.

Histopathologic lesions (Table 18) included a variety of naturally occurring lesions, usually mild. None of the lesions were related to the NC feeding.

Because there were no effects from feeding NC for 24 months, the necropsy on the recovery dogs was omitted after discussion with the technical monitor.

E. Discussion and Conclusions

The various effects observed from these dogs were consistent with maturation and natural variation, except for the increased feed consumption. Such an increase would be the expected outcome of the addition of nonnutritive bulk to the diet. An earlier study in rats[6] found the ^{14}C-labeled NC was not absorbed. It is probable that this same nonabsorption and nontoxicity would be observed in all nonruminant mammals.

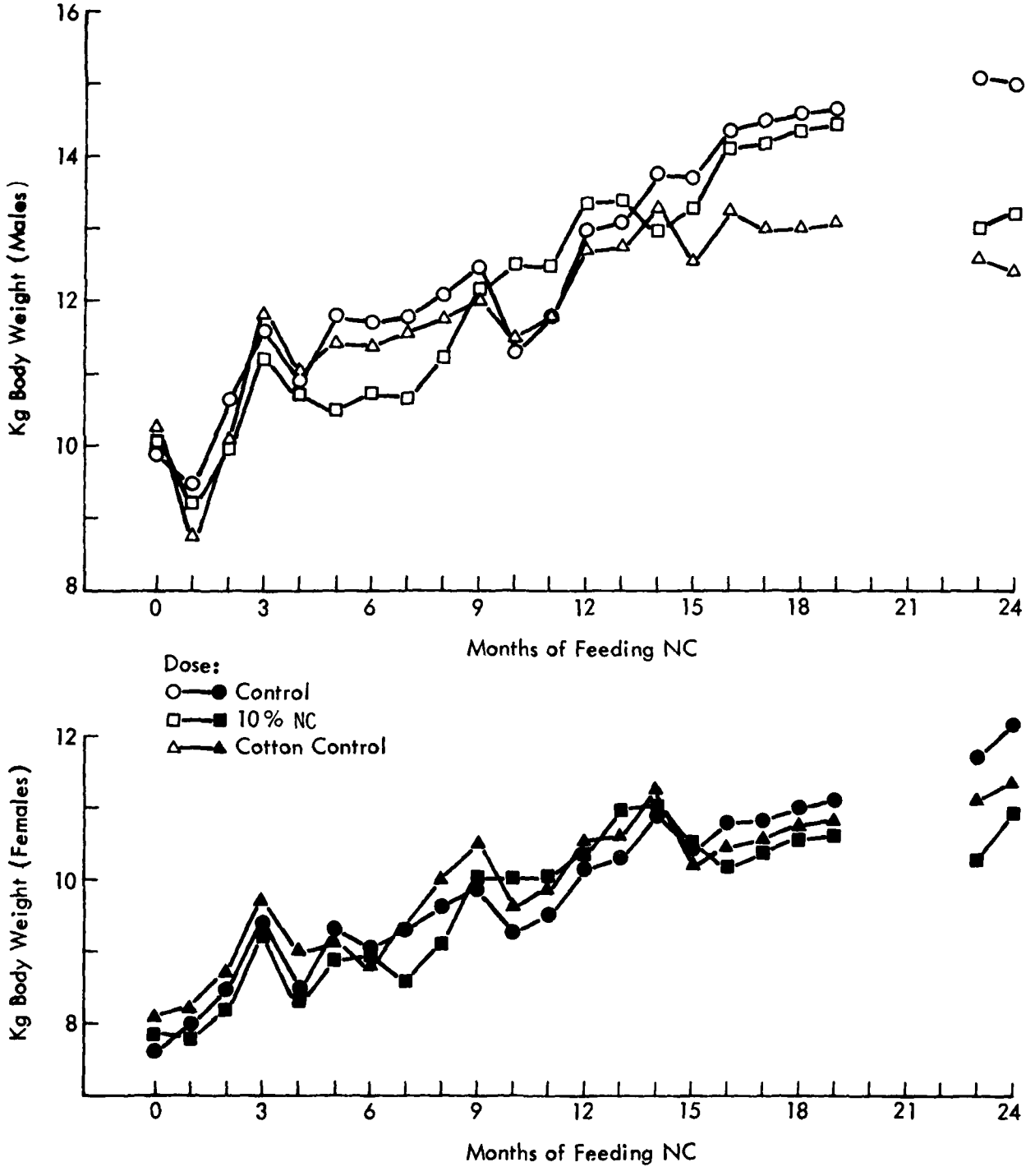

Figure 2 - Average Body Weight of Dogs Fed NC

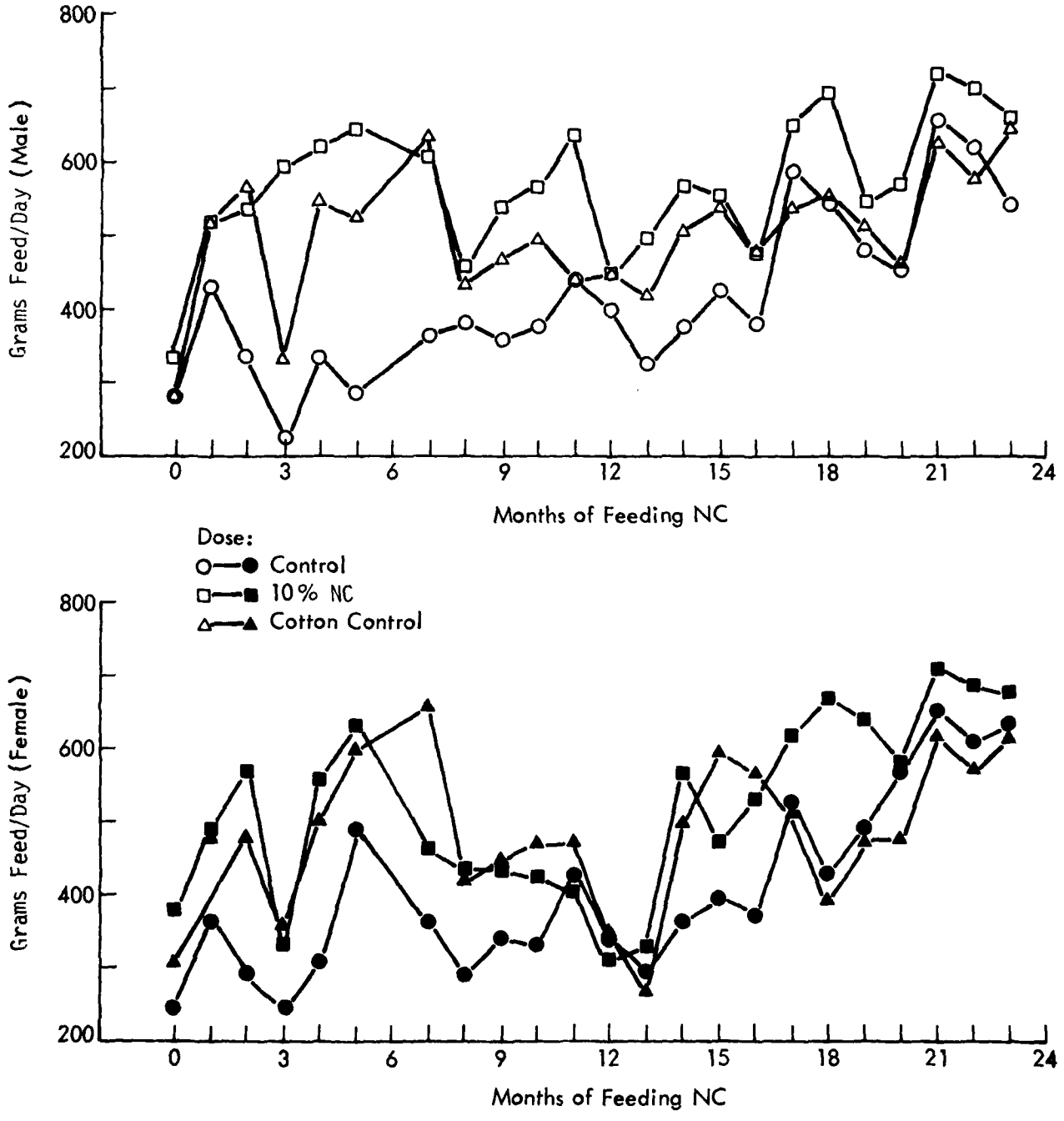

Figure 3 - Average Feed Consumption of Dogs Fed NC

Table 2

AVERAGE FEED CONSUMPTION OF DOGS FED NC

Dose (% in feed)	Feed Consumption, g/dog/day	
	Males	Females
0	422 ± 24[a/]	411 ± 26
1	463 ± 24	428 ± 25
3	456 ± 23	429 ± 24
10	582 ± 17	524 ± 26
10 C[b/]	514 ± 17	492 ± 21

a/ Mean ± standard error of 22 monthly measurements.
b/ Fed 10% cotton linters.

TABLE 3

LABORATORY DATA OF MALE DOGS BEFORE FEEDING OF NITROCELLULOSE

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: % In Feed	0 (C, 6)	1 (T, 6)	3 (T, 6)	10 (T, 6)	10C3/ (C, 6)
ERYTHROCYTES (X10^6 /MM^3)	5.73 ± .15	5.64 ± .10	5.74 ± .13	5.18 ± .22	5.64 ± .17
HEINZ BODIES, %	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0	0.0 + 0.0
RETICULOCYTES, %	.82 ± .11	.77 ± .06	.90 ± .16	.82 ± .06	.99 ± .10
HEMATOCRIT, VOL. %	43.8 ± 1.0	43.1 ± .6	44.8 ± .6	43.0 ± 1.0	43.7 ± .3
HEMOGLOBIN, GM. %	14.7 ± .4	14.6 ± .2	15.1 ± .3	14.7 ± .3	14.7 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 + 0.0
MCV, CUBIC MICRONS	76.5 ± 1.7	76.5 ± .9	78.2 ± 1.4	83.5 ± 2.8	77.7 ± 2.2
MCHH, MICRO MICROGMS.	25.6 ± .6	25.8 ± .3	26.4 ± .6	28.5 ± 1.0[b/]	26.2 ± .8
MCHHC, GM %	33.5 ± .2	33.8 ± .2	33.8 ± .2	34.2 ± .4	33.7 ± .2
PLATELETS (X10^5 /MM^3)	2.5 ± .2	2.4 ± .1	2.3 ± .2	2.6 ± .3	2.7 ± .3
LEUKOCYTES (X10^3 /MM^3)	13.6 ± .6	12.7 ± .9	13.2 ± 1.1	16.9 ± 1.1	14.6 ± 1.1
NEUTROPHILS, %	73.7 ± 2.3	67.1 ± 1.3	63.8 ± 2.8[b/]	65.4 ± 2.6	66.4 ± 2.8
LYMPHOCYTES, %	22.0 ± 1.8	29.1 ± 1.2	31.6 ± 2.9[b/]	27.8 ± 2.2	26.3 ± 1.7
BANDS, %	.3 ± .1	.1 ± .1	.3 ± .3	.9 ± .4	.3 ± .2
MONOCYTES, %	2.8 ± .6	2.3 ± .4	3.0 ± .4	3.8 ± .5	2.8 ± .4
EOSINOPHILS, %	1.3 ± .5	1.5 ± .3	1.3 ± .3	2.1 ± .8	4.2 ± 1.4[b/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.2 ± .2	.1 ± .1	0.0 ± 0.0	.2 ± .1	.2 ± .1
CLOTTING TIME, MIN.	10.1 + .7	9.3 + .4	10.0 + .6	10.0 + .6	9.9 + .4
GLUCOSE (FASTING), MG %	100.5 ± .9	101.5 ± 1.3	101.8 ± .9	101.2 ± .8	101.4 ± .9
SGOT, IU/L	24.8 ± .9	25.1 ± 1.2	23.4 ± .7	24.6 ± 2.4	24.0 ± .5
SGPT, IU/L	34.3 ± .9	32.9 ± 1.2	30.9 ± 1.1	33.3 ± 2.4	35.2 ± 2.5
ALK. PHOS., IU/L	65 ± 3	73 ± 7	63 ± 7	65 ± 4	73 ± 8
BUN, MG %	12.3 ± .9	13.7 ± 2.1	11.7 ± .9	12.7 ± 1.0	12.4 ± 1.3
IMMUNOGLOBIN E, IU/ML	2204 + 271			1683 + 137	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 4

LABORATORY DATA OF FEMALE DOGS BEFORE FEEDING OF NITROCELLULOSE

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0 (C, 6)	1 (T, 6)	3 (T, 6)	10 (T, 6)	10Ca/ (C, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.78 ± .18	5.59 ± .14	5.72 ± .18	5.77 ± .10	5.79 ± .16
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.99 ± .11	1.08 ± .04	.86 ± .08	1.07 ± .06	.90 ± .09
HEMATOCRIT, VOL. %	44.8 ± .9	43.9 ± 1.1	45.5 ± .8	45.7 ± .7	44.2 ± 1.1
HEMOGLOBIN, GM. %	15.0 ± .3	14.7 ± .4	15.2 ± .3	15.3 ± .3	14.8 ± .4
METHEMOGLOBIN, %	1.2 ± .8	0.0 ± 0.0	.8 ± .4	.4 ± .3	2.1 ± .8
MCV, CUBIC MICRONS	77.6 ± 2.0	78.6 ± .9	79.8 ± 1.7	79.2 ± 1.1	76.3 ± .9
MCHB, MICRO MICROGMS.	26.0 ± .8	26.4 ± .3	26.7 ± .6	26.6 ± .4	25.6 ± .3
MCHBC, GM %	33.5 ± .2	33.6 ± .1	33.4 ± .2	33.6 ± .1	33.6 ± .1
PLATELETS ($X10^5$ /MM^3)	2.4 ± .2	3.0 ± .2	2.0 ± .2	2.3 ± .4	2.2 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	10.8 ± .6	9.7 ± .8	9.5 ± .3	11.7 ± .8	11.7 ± 1.0
NEUTROPHILS, %	61.8 ± 2.4	60.8 ± 1.3	60.9 ± 1.9	55.1 ± 2.9	62.5 ± 1.6
LYMPHOCYTES, %	33.0 ± 2.1	33.5 ± 1.6	34.8 ± 2.0	39.3 ± 3.0	31.4 ± 1.4
BANDS, %	.3 ± .2	.2 ± .1	.1 ± .1	.1 ± .1	.3 ± .1
MONOCYTES, %	2.3 ± .6	2.0 ± .2	2.3 ± .4	1.8 ± .5	1.5 ± .3
EOSINOPHILS, %	2.7 ± .5	3.5 ± .8	1.9 ± .2	3.8 ± .6	4.3 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.2 ± .1	.1 ± .1	.4 ± .2b/	.1 ± .1
CLOTTING TIME, MIN.	8.0 ± .5	8.9 ± .5	8.3 ± .6	7.8 ± .7	7.2 ± .6
GLUCOSE (FASTING), MG %	96.7 ± 1.1	92.8 ± 1.7	94.7 ± 2.1	92.7 ± 2.0	93.3 ± 1.5
SGOT, IU/L	22.1 ± 1.7	22.8 ± .9	21.9 ± .9	23.3 ± .7	22.2 ± 1.8
SGPT, IU/L	34.0 ± 2.4	36.3 ± 2.1	36.7 ± 2.3	34.3 ± 1.8	33.8 ± 2.5
ALK. PHOS., IU/L	56 ± 6	60 ± 3	68 ± 5	70 ± 3	61 ± 7
BUN, MG %	12.6 ± .9	13.0 ± .7	12.0 ± .6	13.0 ± .8	13.4 ± .8
IMMUNOGLOBIN E, IU/ML	2179 ± 221			1683 ± 137	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 5

LABORATORY DATA OF MALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 3 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % In Feed	0.000 (C, 6)	1.000 (T, 6)	3.000 (T, 6)	10.000 (T, 6)	10.0 a/ (C, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.09 ± .22	4.91 ± .07	5.40 ± .16	4.85 ± .29	5.37 ± .11
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.72 ± .09	.46 ± .07	.59 ± .12	.65 ± .08	.59 ± .08
HEMATOCRIT, VOL. %	41.0 ± 1.4	40.0 ± .3	43.0 ± .7	41.7 ± .6	43.2 ± .7
HEMOGLOBIN, GM. %	13.7 ± .5	13.1 ± .1	14.2 ± .3	13.8 ± .2	14.3 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	80.8 ± 1.3	81.5 ± .9	79.9 ± 1.7	87.6 ± 5.5	80.4 ± 1.2
MCHB, MICRO MICROGMS.	27.0 ± .4	26.8 ± .3	26.4 ± .6	29.2 ± 2.1	26.7 ± .4
MCHBC, GM %	33.4 ± .2	32.9 ± .2	33.1 ± .1	33.3 ± .4	33.2 ± .3
PLATELETS ($X10^5$ /MM^3)	2.8 ± .3	2.6 ± .2	3.1 ± .2	3.7 ± .5	3.0 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	17.0 ± 4.5	11.0 ± .5	10.7 ± .6	15.2 ± .6	13.1 ± 2.0
NEUTROPHILS, %	66.7 ± 4.5	56.5 ± 2.0	57.8 ± 4.0	67.8 ± 2.8	69.7 ± 3.9
LYMPHOCYTES, %	26.7 ± 2.8	32.0 ± 1.8	32.7 ± 4.6	28.5 ± 2.5	22.3 ± 3.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0	.2 ± .2
EOSINOPHILS, %	6.2 ± 1.9	10.7 ± 2.7	7.7 ± 1.0	7.7 ± .7	7.3 ± .7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.8 ± .3	1.7 ± .8	1.0 ± .4	.5 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.4 ± .2	6.8 ± .3	6.6 ± .3	6.5 ± .3	6.8 ± .2
GLUCOSE (FASTING), MG %	7.2 ± 1.2 (5)	5.2 ± 1.3	6.5 ± 1.2 (4)	4.4 ± 1.5 (5)	4.7 ± 1.2
SGOT, IU/L	29.7 ± 3.1	31.0 ± 1.3	27.5 ± 2.1	29.2 ± 2.2	29.8 ± 2.3
SGPT, IU/L	38.0 ± 3.0	44.0 ± 2.5	36.0 ± 3.5	36.3 ± 3.7	36.0 ± 1.8
ALK. PHOS., IU/L	55 ± 11	40 ± 4	32 ± 5	45 ± 4	37 ± 3
CHOLESTEROL, MG %	158 ± 21	150 ± 12	138 ± 5	153 ± 8	155 ± 8
BUN, MG %	12.2 ± 1.9	13.0 ± .5	12.1 ± 1.2	9.7 ± .8	11.9 ± .5
IMMUNOGLOBULIN E, IU/ML	313 ± 198			1000 ± 41	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 6

LABORATORY DATA OF FEMALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 3 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % In Feed	0.000 (C, 6)	1.000 (T, 6)	3.000 (T, 6)	10.000 (T, 6)	Local (C, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.73 ± .26	5.66 ± .16	5.40 ± .15	5.58 ± .13	5.45 ± .27
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.60 ± .14	1.11 ± .30	.80 ± .09	.78 ± .16	.59 ± .12
HEMATOCRIT, VOL, %	42.0 ± 1.7	42.5 ± 1.1	41.7 ± .5	43.0 ± .9	40.2 ± 1.5
HEMOGLOBIN, GM, %	14.6 ± .6	14.8 ± .6	14.5 ± .2	14.8 ± .5	14.0 ± .6
METHEMOGLOBIN, %	1.4 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 1.2	0.0 ± 0.0
MCV, CUBIC MICRONS	73.5 ± 1.2	75.2 ± 1.4	77.3 ± 1.6	77.1 ± 1.2	73.9 ± 1.3
MCHB, MICRO MICROGMS.	25.5 ± .5	26.2 ± .7	26.9 ± .6	26.5 ± .6	25.8 ± .4
MCHBC, GM %	34.7 ± .1	34.8 ± .5	34.7 ± .4	34.4 ± .5	35.0 ± .3
PLATELETS ($X10^5$ /MM^3)	2.7 ± .2	3.6 ± .2 [b/]	2.8 ± .3	2.7 ± .1	3.0 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	15.0 ± 1.4	13.7 ± 1.4	11.9 ± .8	14.1 ± .9	15.0 ± 1.0
NEUTROPHILS, %	68.0 ± 1.9	66.8 ± 3.9	64.0 ± 3.7	61.7 ± 2.0	66.3 ± 1.5
LYMPHOCYTES, %	23.8 ± 2.1	28.3 ± 3.4	31.8 ± 2.6	32.8 ± 2.8	27.7 ± 2.4
BANDS, %	.2 ± .2	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0	.2 ± .2
EOSINOPHILS, %	6.7 ± 2.3	3.3 ± .9	3.3 ± .8	2.8 ± .9	3.7 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .5	1.5 ± .8	1.5 ± .6	2.7 ± 1.9	2.2 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.4 ± .5	5.9 ± .4	6.8 ± .3	6.3 ± .2	6.6 ± .9
GLUCOSE (FASTING), MG %	6.7 ± 1.1	2.8 ± .8 (4)	6.2 ± 1.1	6.2 ± 1.2	5.2 ± 1.7 (5)
SGOT, IU/L	24.8 ± 1.1	29.2 ± 2.2	24.8 ± 1.1	27.2 ± 1.1	27.7 ± 1.3
SGPT, IU/L	36.3 ± 4.3	32.0 ± .6	35.0 ± 1.8	33.0 ± 2.3	34.5 ± 2.0
ALK. PHOS., IU/L	39 ± 4	37 ± 3	45 ± 6	49 ± 6	35 ± 4
CHOLESTEROL, MG %	138 ± 9	139 ± 9	158 ± 11	158 ± 12	151 ± 15
BUN, MG %	14.1 ± .8	13.2 ± 1.7	12.7 ± 1.5	13.8 ± 1.3	11.4 ± .7
IMMUNOGLOBULIN E, IU/ML	367 ± 232			1067 ± 43	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 7

LABORATORY DATA OF MALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 9 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0.000 (C, 6)	1.000 (T, 6)	3.000 (T, 6)	10.000 (T, 6)	10.0a/ (C, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.22 ± .24	6.03 ± .15	6.40 ± .17	6.33 ± .14	6.36 ± .15
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.44 ± .05	.43 ± .06	.52 ± .13	.49 ± .05	.44 ± .05
HEMATOCRIT, VOL. %	43.8 ± 1.1	43.0 ± 1.1	46.2 ± 1.2	45.8 ± .8	44.2 ± 1.4
HEMOGLOBIN, GM. %	15.4 ± .4	14.6 ± .3	15.5 ± .4	15.9 ± .2	15.4 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	70.8 ± 2.0	71.3 ± .6	72.2 ± 1.5	72.5 ± 1.1	69.4 ± .8
MCHB, MICRO MICROGMS.	24.9 ± .4	24.2 ± .2	24.2 ± .2	25.2 ± .4	24.3 ± .3
MCHBC, GM %	35.2 ± .6	33.9 ± .2	33.6 ± .6 b/	34.8 ± .4	34.9 ± .2
PLATELETS ($X10^5$ /MM^3)	2.4 ± .2	2.2 ± .1	2.6 ± .2	2.8 ± .2	2.5 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	13.4 ± 1.7	11.5 ± .6	12.2 ± .3	12.7 ± .4	10.8 ± .6
NEUTROPHILS, %	72.2 ± 3.9	63.3 ± 1.9	66.3 ± 3.1	61.8 ± 2.0	69.5 ± 2.4
LYMPHOCYTES, %	23.7 ± 3.7	29.7 ± 2.0	27.0 ± 3.9	31.5 ± 2.5	27.2 ± 2.0
BANDS, %	0.0 ± 0.0	.2 ± .2	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.8 ± .9	5.8 ± 1.2	5.8 ± 1.7	4.8 ± .7	4.3 ± 1.3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.3 ± .6	1.0 ± .5	.7 ± .4	1.8 ± .7	.5 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.0 ± .4	9.8 ± .5 b/	9.2 ± .9	8.4 ± .2	7.0 ± .9
GLUCOSE (FASTING), MG %	93.7 ± 1.9	90.7 ± 2.1	93.0 ± 3.3	90.7 ± 3.2	94.5 ± 1.1
SGOT, IU/L	27.8 ± 3.9	22.8 ± 2.8	20.7 ± 1.8	22.7 ± 2.7	22.7 ± 1.4
SGPT, IU/L	33.8 ± 4.3	34.7 ± 4.0	31.3 ± 3.1	36.5 ± 7.5	35.5 ± 2.0
ALK. PHOS., IU/L	48 ± 9	39 ± 6	28 ± 4	37 ± 5	33 ± 4
CHOLESTEROL, MG %	146 ± 9	141 ± 9	126 ± 4	137 ± 8	135 ± 6
BUN, MG %	11.7 ± .6	11.7 ± .7	10.8 ± .5	9.5 ± .6	11.7 ± .8
IMMUNOGLOBULIN E, IU/ML				1621 ± 32	1758 ± 72

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 8

LABORATORY DATA OF FEMALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 9 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0.000 (C, 6)	1.000 (T, 6)	3.000 (T, 6)	10.000 (T, 6)	10[a] (C, 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.61 ± .25	6.24 ± .30	6.01 ± .33	5.98 ± .26	6.05 ± .16
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.49 ± .07	.56 ± .16	.60 ± .06	.56 ± .10	1.02 ± .14[b]
HEMATOCRIT, VOL. %	47.7 ± 1.9	46.2 ± 1.9	46.0 ± 1.6	45.0 ± 1.6	44.8 ± .9
HEMOGLOBIN, GM. %	16.7 ± .5	15.7 ± .6	15.6 ± .5	15.4 ± .6	15.5 ± .3
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± .7	.5 ± .5
MCV, CUBIC MICRONS	72.1 ± .7	74.2 ± .9	76.9 ± 1.6	75.4 ± 1.5	74.3 ± 1.7
MCHB, MICRO MICROGMS.	25.3 ± .3	25.3 ± .4	26.1 ± .6	25.8 ± .5	25.7 ± .6
MCHBC, GM %	35.0 ± .4	34.1 ± .2	33.9 ± .4[b]	34.2 ± .3	34.6 ± .1
PLATELETS ($X10^5$ /MM^3)	3.0 ± .3	2.7 ± .3	2.6 ± .3	2.7 ± .3	2.5 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	11.2 ± .9	9.2 ± .5	10.1 ± 1.1	9.5 ± .9	11.3 ± .5
NEUTROPHILS, %	67.3 ± 2.4	59.7 ± 3.1	62.5 ± 4.0	61.8 ± 3.4	66.8 ± 2.4
LYMPHOCYTES, %	27.8 ± 2.2	34.7 ± 3.4	30.3 ± 3.4	35.5 ± 3.6	27.3 ± 2.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.8 ± 1.1	4.7 ± 1.0	6.5 ± .9	2.5 ± .8	5.2 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .4	1.0 ± .4	.7 ± .3	.2 ± .2	.7 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.0 ± .5	6.3 ± .9	6.6 ± .4	5.6 ± .2	6.0 ± .2
GLUCOSE (FASTING), MG %	86.3 ± 2.3	89.7 ± 2.8	88.5 ± 2.2	89.2 ± 1.1	82.5 ± 2.2
SGOT, IU/L	20.7 ± 2.1	16.7 ± 2.7	20.5 ± 1.4	21.2 ± 2.0	24.3 ± 1.3
SGPT, IU/L	34.3 ± 3.6	27.7 ± 1.9	35.0 ± 1.8	35.5 ± 3.5	35.0 ± 2.8
ALK. PHOS., IU/L	43 ± 7	34 ± 5	50 ± 7	49 ± 8	41 ± 9
CHOLESTEROL, MG %	151 ± 17	140 ± 18	148 ± 13	138 ± 15	147 ± 10
BUN, MG %	13.8 ± 1.0	14.5 ± .6	12.3 ± 1.1	14.8 ± 1.3	13.2 ± .6
IMMUNOGLOBULIN E, IU/ML				1471 ± 60	1646 ± 69

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 9

LABORATORY DATA OF MALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 12 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: % In Feed	0.000 (C. 6)	1.000 (T. 6)	3.000 (T. 6)	10.000 (T. 6)	10.0[a/] (C. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	6.28 ± .26	6.06 ± .14	6.20 ± .16	6.17 ± .12	6.64 ± .13
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.64 ± .11	.49 ± .09	.36 ± .05	.42 ± .04	.59 ± .11
HEMATOCRIT, VOL. %	45.5 ± 1.2	44.0 ± .8	44.3 ± 1.1	46.0 ± .8	47.5 ± .9
HEMOGLOBIN, GM. %	15.8 ± .4	15.0 ± .3	15.3 ± .4	16.0 ± .3	16.5 ± .3
METHEMOGLOBIN, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
MCV, CUBIC MICRONS	72.7 ± 1.3	72.7 ± .9	71.5 ± 1.0	74.6 ± 1.0	71.5 ± .7
MCHB, MICRO MICROGMS.	25.2 ± .6	24.8 ± .2	24.8 ± .6	26.0 ± .3	24.8 ± .2
MCHBC, GM %	34.7 ± .3	34.2 ± .2	34.6 ± .2	34.9 ± .2	34.7 ± .1
PLATELETS ($X10^5$ /MM^3)	2.4 ± .2	2.5 ± .2	2.7 ± .1	2.8 ± .2	2.8 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	11.1 ± 1.0	8.7 ± .4	9.4 ± .6	11.7 ± .6	9.6 ± .5
NEUTROPHILS, %	62.5 ± 2.4	54.5 ± 2.8	59.7 ± 2.5	53.2 ± 3.2	59.7 ± 4.0
LYMPHOCYTES, %	34.0 ± 1.8	39.5 ± 2.1	31.8 ± 2.2	43.0 ± 3.4	33.2 ± 2.7
BANDS, %	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.0 ± 1.3	5.5 ± 1.1	8.3 ± 1.1	3.7 ± 1.0	6.8 ± 1.6
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .2	.2 ± .2	.2 ± .2	.3 ± .2
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	.2 ± .2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	6.8 ± .3	6.4 ± .5	7.3 ± .4	6.4 ± .3	6.6 ± .3
GLUCOSE (FASTING), MG %	4.5 ± 1.2	3.7 ± 1.1	6.2 ± .9	10.2 ± 3.0	5.0 ± 1.0
SGOT, IU/L	33.0 ± 1.8	30.3 ± 1.9	30.0 ± 1.3	27.5 ± 1.6	31.5 ± .9
SGPT, IU/L	55.0 ± 13.8	39.3 ± 4.2	34.7 ± 3.8	39.3 ± 6.4	36.0 ± 2.5
ALK. PHOS., IU/L	36 ± 4	30 ± 4	24 ± 3	32 ± 5	28 ± 3
CHOLESTEROL, MG %	133 ± 4	130 ± 11	121 ± 3	129 ± 8	132 ± 6
BUN, MG %	12.7 ± .6	12.5 ± .8	11.0 ± 1.2	12.2 ± .5	12.0 ± .7
IMMUNOGLOBULIN E, IU/ML	3017 ± 225			1913 ± 183[b/]	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 10

LABORATORY DATA OF FEMALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 12 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0.000 (C. 6)	1.000 (T. 6)	3.000 (T. 6)	10.000 (T. 6)	10[a/] (C. 6)
ERYTHROCYTES ($X10^6$ /MM^3)	5.99 ± .18	5.95 ± .26	5.70 ± .25	5.89 ± .17	6.17 ± .19
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.43 ± .07	.43 ± .09	.51 ± .15	.29 ± .05	.39 ± .08
HEMATOCRIT, VOL. %	44.5 ± 1.6	45.5 ± 1.3	43.2 ± 1.7	44.3 ± 1.3	46.2 ± 1.2
HEMOGLOBIN, GM. %	15.6 ± .5	15.9 ± .5	15.2 ± .5	15.4 ± .5	16.1 ± .4
METHEMOGLOBIN, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	74.2 ± 1.1	76.7 ± 1.6	75.8 ± .8	75.3 ± .7	75.0 ± 1.2
MCHB, MICRO MICROGMS.	26.1 ± .3	26.8 ± .7	26.7 ± .4	26.1 ± .2	26.2 ± .4
MCHBC, GM %	35.1 ± .2	34.9 ± .2	35.2 ± .3	34.7 ± .3	34.9 ± .1
PLATELETS ($X10^5$ /MM^3)	2.7 ± .2	3.0 ± .2	2.5 ± .2	3.1 ± .2	2.5 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	13.3 ± 2.5	10.4 ± .8	9.7 ± .8	11.5 ± 1.1	9.6 ± .6
NEUTROPHILS, %	61.8 ± 2.7	64.8 ± 4.0	64.5 ± 1.5	58.3 ± 1.8	64.0 ± 3.6
LYMPHOCYTES, %	32.3 ± 2.3	29.8 ± 3.0	31.0 ± 1.5	38.2 ± 1.8	31.0 ± 3.8
BANDS, %	.2 ± .2	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0	.2 ± .2
EOSINOPHILS, %	4.0 ± 1.2	4.0 ± 1.2	3.7 ± .9	3.2 ± .6	3.7 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.7 ± .9	1.3 ± .6	.7 ± .3	.3 ± .2	1.2 ± .4
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.9 ± .4	7.8 ± .4	7.9 ± .5	7.9 ± .1	9.7 ± .6[b/]
GLUCOSE (FASTING), MG %	7.0 ± 1.0 (3)	5.2 ± 1.6 (5)	5.0 ± 1.3 (5)	4.3 ± 1.0	3.3 ± .4
SGOT, IU/L	23.7 ± 2.2	21.0 ± 1.5	20.0 ± 1.0	22.7 ± 1.2	24.5 ± 3.1
SGPT, IU/L	28.0 ± 3.4	28.2 ± 3.7	33.0 ± 2.3	31.2 ± 4.5	33.0 ± 1.5
ALK. PHOS., IU/L	36 ± 4	28 ± 3	44 ± 9	38 ± 7	32 ± 5
CHOLESTEROL, MG %	178 ± 19	153 ± 21	182 ± 24	178 ± 19	175 ± 18
BUN, MG %	11.4 ± .3	10.7 ± .7	11.8 ± 1.4	15.7 ± 1.1[b]	12.5 ± .7
IMMUNOGLOBULIN E, IU/ML	2821 ± 211			1979 ± 205[b/]	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE II

LABORATORY DATA OF MALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0 (C, 3)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10Ca/ (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.65 ± .11	6.07 ± .12	6.17 ± .35	6.67 ± .19	6.27 ± .16
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.67 ± .13	.61 ± .11	.76 ± .10	.66 ± .15	.46 ± .04
HEMATOCRIT, VOL. %	48.3 ± .9	45.5 ± .3	45.8 ± 2.2	50.5 ± 1.7	45.8 ± 1.1
HEMOGLOBIN, GM. %	16.4 ± .1	14.7 ± .3	15.0 ± .9	17.0 ± .3	15.1 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	72.7 ± 2.4	75.0 ± 1.6	74.2 ± 1.1	75.7 ± .9	73.0 ± .5
MCHB, MICRO MICROGMS.	24.6 ± .5	24.3 ± .7	24.2 ± .3	25.5 ± .3	24.1 ± .4
MCHBC, GM %	33.9 ± .4	32.4 ± .5	32.7 ± .5	33.7 ± .6	33.0 ± .5
PLATELETS ($X10^5$ /MM^3)	2.8 ± .4	2.7 ± .3	2.8 ± .1	2.6 ± .2	2.5 ± .1
LEUKOCYTES ($X10^3$ /MM^3)	11.0 ± 1.0	9.9 ± .6	11.2 ± 1.5	10.4 ± .3	9.7 ± .8
NEUTROPHILS, %	62.7 ± 6.2	57.8 ± 4.3	64.0 ± 2.8	55.0 ± 1.8	63.5 ± 3.2
LYMPHOCYTES, %	31.0 ± 4.0	37.3 ± 3.2	30.5 ± 3.5	40.5 ± 2.3	30.0 ± 3.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
EOSINOPHILS, %	4.0 ± 1.5	3.8 ± 2.5	4.3 ± 1.3	3.3 ± 1.3	5.8 ± 1.1
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.3 ± .9	1.3 ± .3	1.3 ± .8	1.3 ± .8	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	1.0 ± 1.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	7.5 ± .3	6.3 ± .3	5.8 ± .3	7.6 ± 1.0	7.4 ± .3
GLUCOSE (FASTING), MG %	4.3 ± 1.9	4.7 ± .3 (3)	6.0 ± 1.7	3.3 ± 1.9	2.8 ± 1.1
SGOT, IU/L	23.3 ± 2.3	34.0 ± 2.7	86.3 ± 59.9	21.0 ± 1.2	33.8 ± 6.5
SGPT, IU/L	29.0 ± 1.0	37.8 ± 5.0	51.8 ± 17.9	33.0 ± 4.5	42.5 ± 9.5
ALK. PHOS., IU/L	22 ± 4	17 ± 2	35 ± 16	29 ± 3	34 ± 15
CHOLESTEROL, MG %	139 ± 10	142 ± 12	148 ± 17	160 ± 14	150 ± 3
BUN, MG %	10.6 ± 1.2	14.5 ± .6[b/]	11.6 ± .4	12.5 ± .5	14.6 ± 1.0[b/]
IMMUNOGLOBULIN E, IU/ML	1350 ± 427			1463 ± 163	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 17

LABORATORY DATA OF FEMALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10a/ (C, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	5.44 ± .19	6.20 ± .34	5.77 ± .18	6.22 ± .18	5.71 ± .30
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.98 ± .27	1.01 ± .15	.34 ± .05 b/	.88 ± .13	.82 ± .12
HEMATOCRIT, VOL, %	43.3 ± .3	46.8 ± 1.8	45.5 ± 1.7	48.3 ± 3.0	43.3 ± 1.7
HEMOGLOBIN, GM, %	14.0 ± .2	15.1 ± .4	15.0 ± .7	15.8 ± .9 (3)	14.1 ± .6
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	79.8 ± 2.6	75.5 ± 1.3	78.9 ± 1.3	77.3 ± 2.9	75.9 ± 1.4
MCHB, MICRO MICROGMS,	25.9 ± .5	24.4 ± .7	26.0 ± .8	25.7 ± .7 (3)	24.7 ± .5
MCHBC, GM %	32.5 ± .4	32.3 ± .4	33.0 ± .6	34.0 ± .8 (3)	32.6 ± .2
PLATELETS ($\times 10^5$ /MM^3)	2.9 ± .2	3.0 ± .2	2.8 ± .3	2.9 ± .4	2.8 ± .4
LEUKOCYTES ($\times 10^3$ /MM^3)	9.4 ± 1.5	10.2 ± .6	10.4 ± .9	11.2 ± 1.7	7.9 ± .3
NEUTROPHILS, %	57.8 ± 3.9	53.5 ± 3.5	57.5 ± 4.8	54.0 ± 4.0	55.3 ± 3.0
LYMPHOCYTES, %	36.0 ± 5.1	41.5 ± 2.9	35.0 ± 5.1	42.5 ± 5.6	37.3 ± 5.1
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	5.5 ± 1.7	4.3 ± 1.6	5.3 ± 2.4	3.0 ± 1.6	6.3 ± 2.7
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .5	.8 ± .5	2.0 ± .4	.5 ± .5	1.3 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
CLOTTING TIME, MIN,	7.5 ± .4	6.4 ± .2	6.9 ± .1	7.8 ± .4	7.1 ± .5
GLUCOSE (FASTING), MG %	5.3 ± 1.6	5.3 ± 1.5	5.3 ± 2.5	6.3 ± 1.4	5.8 ± .6
SGOT, IU/L	22.5 ± .9	24.3 ± 1.4	23.5 ± 1.7	21.0 ± 1.2	24.5 ± 3.0
SGPT, IU/L	26.0 ± 3.1	26.3 ± 1.8	31.8 ± 2.3	24.3 ± 2.7	28.3 ± 3.6
ALK, PHOS., IU/L	35 ± 5	26 ± 5	31 ± 4	26 ± 7	29 ± 4
CHOLESTEROL, MG %	194 ± 19	167 ± 21	220 ± 24	180 ± 15	185 ± 29
BUN, MG %	13.5 ± 1.1	11.8 ± 1.2	12.8 ± 1.3	12.7 ± 1.3	12.3 ± .6
IMMUNOGLOBULIN E, IU/ML	1213 ± 247			1625 ± 431	

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 13

LABORATORY DATA OF MALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0.00 (C. 3)	1.00 (T. 3)	3.00 (T. 4)	10.00 (T. 4)	10.0a/ (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.01 ± .39	6.47 ± .24	6.58 ± .35	6.40 ± .08	6.90 ± .16
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.42 ± .12	.41 ± .04	.47 ± .09	.38 ± .03	.37 ± .08
HEMATOCRIT, VOL. %	51.0 ± 1.7	47.0 ± 1.0	48.5 ± 2.0	49.8 ± .6	51.0 ± 1.7
HEMOGLOBIN, GM. %	16.9 ± .7	15.7 ± .6	15.7 ± .7	16.3 ± .3	16.8 ± .5
METHEMOGLOBIN, %	1.3 ± .8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	73.0 ± 1.7	72.8 ± 1.1	73.8 ± 1.0	77.7 ± 1.2	73.8 ± 1.1
MCHB, MICRO MICROGMS.	24.2 ± .5	24.2 ± .1	23.9 ± .4	25.5 ± .7	24.3 ± .4
MCHBC, GM %	33.1 ± .3	33.3 ± .5	32.4 ± .5	32.8 ± .5	32.9 ± .2
PLATELETS ($X10^5$ /MM^3)	2.4 ± .1	2.4 ± .4	2.7 ± .1	3.1 ± .1	2.7 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	11.4 ± .6	11.0 ± .3	11.8 ± 1.6	12.3 ± .9	10.5 ± 1.1
NEUTROPHILS, %	65.0 ± 4.0	60.7 ± 4.4	70.8 ± 4.5	66.0 ± 1.6	67.0 ± 3.2
LYMPHOCYTES, %	33.0 ± 4.7	34.7 ± 5.5	25.8 ± 3.1	32.8 ± 1.7	29.8 ± 2.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
EOSINOPHILS, %	1.7 ± .9	4.3 ± 1.2	3.3 ± 1.4	1.0 ± .6	2.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.3 ± .3	.3 ± .3	0.0 ± 0.0	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
CLOTTING TIME, MIN.	10.2 ± .9	10.8 ± .3	10.6 ± .8	9.1 ± .3	7.9 ± 1.4
GLUCOSE (FASTING), MG %	4.7 ± 2.0	4.7 ± 2.2	4.3 ± 2.0 (3)	6.0 ± 1.7 (3)	6.5 ± .5 (2)
SGOT, IU/L	21.0 ± 0.0	19.0 ± 2.6	23.3 ± .8	18.0 ± 1.7	25.8 ± 2.8
SGPT, IU/L	29.0 ± 1.0	29.7 ± 6.4	32.3 ± 6.5	40.8 ± 5.8	31.8 ± 1.4
ALK. PHOS., IU/L	24 ± 1	28 ± 5	36 ± 11	34 ± 11	25 ± 4
BSP, %	3.0 ± 1.0 (2)	5.0 ± 2.0 (2)	6.5 ± 1.5 (2)	6.0 ± 2.0 (2)	5.5 ± .5 (2)
CHOLESTEROL, MG %	133 ± 3	145 ± 14	133 ± 1	135 ± 13	147 ± 8
BUN, MG %	9.7 ± 1.4	11.3 ± .6	9.5 ± .9	15.0 ± 3.0	12.4 ± 1.6
IMMUNOGLOBULIN E, IU/ML	2700 ±1106				2075 ±1022

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 14

LABORATORY DATA OF FEMALE DOGS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF DOGS

DOSE: % in Feed	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10[a] (C, 4)
ERYTHROCYTES ($\times 10^6$ /MM^3)	6.47 ± .32	6.46 ± .21	6.47 ± .20	6.38 ± .17	6.55 ± .18
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.48 ± .07	.50 ± .10	.42 ± .04	.76 ± .28	.54 ± .06
HEMATOCRIT, VOL. %	49.8 ± 2.5	48.5 ± 1.0	49.3 ± 1.4	50.0 ± 1.4	49.3 ± 1.7
HEMOGLOBIN, GM. %	16.3 ± .6	16.0 ± .4	16.2 ± .5	16.5 ± .5	16.2 ± .5
METHEMOGLOBIN, %	.6 ± .4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	76.9 ± 1.1	75.1 ± 1.3	76.2 ± 1.3	78.4 ± 1.2	75.1 ± .9
MCHH, MICRO MICROGMS.	25.3 ± .3	24.9 ± .5	25.1 ± .5	25.9 ± .5	24.8 ± .4
MCHHC, GM %	32.9 ± .4	33.1 ± .1	32.9 ± .2	33.0 ± .3	33.0 ± .3
PLATELETS ($\times 10^5$ /MM^3)	3.2 ± .4	2.8 ± .4	2.9 ± .3	2.9 ± .3	3.0 ± .3
LEUKOCYTES ($\times 10^3$ /MM^3)	12.0 ± 1.0	10.9 ± .9	8.7 ± .5[b]	12.5 ± .8	10.4 ± .4
NEUTROPHILS, %	69.8 ± .5	60.5 ± 3.8	64.0 ± 2.6	64.8 ± 3.3	62.8 ± 4.5
LYMPHOCYTES, %	28.0 ± 1.5	38.8 ± 3.2	34.8 ± 2.7	33.3 ± 3.5	33.8 ± 4.0
BANDS, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.5 ± .9	.8 ± .8	1.3 ± .3	2.0 ± 1.4	3.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0	0.0 ± 0.0
CLOTTING TIME, MIN.	8.8 ± .6	9.0 ± .4	8.6 ± 1.0	8.4 ± .5	9.3 ± .1
GLUCOSE (FASTING), MG %	5.8 ± 1.3	6.0 ± 1.0 (2)	4.3 ± 1.7 (3)	4.7 ± 1.2 (3)	4.5 ± 1.2
SGOT, IU/L	22.5 ± .9	23.3 ± .3	19.5 ± 1.9	22.0 ± 2.4	20.3 ± 1.9
SGPT, IU/L	26.8 ± 1.7	24.3 ± 2.4	35.5 ± 1.9	33.0 ± 5.1	27.8 ± 2.7
ALK. PHOS., IU/L	44 ± 6	33 ± 6	33 ± 5	40 ± 4	29 ± 4
BSP, %	5.0 ± 1.0 (2)	3.0 ± 0.0 (2)	7.5 ± 2.5 (2)	5.5 ± 1.5 (2)	8.5 ± .5 (2)
CHOLESTEROL, MG %	166 ± 17	136 ± 18	143 ± 9	149 ± 16	134 ± 6
BUN, MG %	9.8 ± .5	9.1 ± .8	11.0 ± .7	12.4 ± 1.0	9.3 ± .5
IMMUNOGLOBULIN E, IU/ML	1388 ± 357			875 ± 189	963 ± 369

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL DOGS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 15

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS FED NC FOR 12 MONTHS

Dose (% in feed)	Dog No.	Terminal body weight (kg)	Absolute Organ Weight (gm)									
			Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	51	12.4	84.5	101.0	385.0	62.9	78.0	1.03	0.09	0.84	13.7	
0	52	10.0	77.3	60.1	304.2	50.9	55.5	1.61	0.08	0.49		1.36
1	59	13.1	80.9	107.7	401.7	65.6	53.5	2.15	0.08	1.22	19.2	
1	60	10.8	94.9	91.8	302.1	57.3	135.8	2.14	0.08	0.88		1.24
3	63	10.6	94.0	92.4	347.9	61.6	38.4	1.80	0.07	0.99	15.7	
3	64	11.9	90.4	92.6	400.6	53.3	37.3	1.69	0.08	1.07		1.62
10	67	9.6	76.9	72.4	299.8	57.2	44.4	1.88	0.08	0.90	22.6	
10	68	9.7	85.6	72.1	333.2	63.0	75.7	2.17	0.07	0.74		2.88
10C[a/]	55	11.8	79.5	83.9	346.9	67.6	68.6	1.39	0.06	0.83	19.2	
10C	56	10.2	68.2	73.7	339.9	53.3	110.5	1.83	0.08	0.99		2.32

Dose (% in feed)	Dog No.	Relative Organ Weight (gm/kg body weight)									
		Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	51	6.81	8.15	31.0	5.07	6.29	0.140	0.007	0.071	1.10	
0	52	7.73	6.01	30.4	5.09	5.55	0.161	0.008	0.049		0.136
1	59	6.18	8.18	30.7	5.01	4.08	0.164	0.006	0.093	1.47	
1	60	8.79	8.50	30.0	5.31	12.57	0.198	0.007	0.081		0.115
3	63	8.70	8.56	32.2	5.70	3.56	0.167	0.006	0.092	1.45	
3	64	7.60	7.78	33.7	4.48	3.13	0.142	0.007	0.090		0.136
10	67	8.22	7.54	31.2	5.96	4.63	0.196	0.009	0.094	2.35	
10	68	8.82	7.43	34.4	6.49	7.80	0.224	0.007	0.076		0.297
10C	55	6.74	7.11	29.4	5.75	5.81	0.118	0.005	0.070	1.40	
10C	56	6.69	7.23	33.3	5.23	10.83	0.179	0.008	0.097		0.227

Dose (% in feed)	Dog No.	Relative Organ Weight (gm/gm brain weight)								
		Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	51	1.195	4.56	0.744	0.92	0.0205	0.0011	0.0105	0.162	
0	52	0.777	3.94	0.658	0.72	0.0208	0.0010	0.0063		0.0175
1	59	1.331	4.97	0.811	0.66	0.0266	0.0010	0.0151	0.237	
1	60	0.967	3.18	0.604	1.43	0.0226	0.0008	0.0092		0.0131
3	63	0.983	3.70	0.655	0.41	0.0191	0.0007	0.0105	0.167	
3	64	1.024	4.43	0.590	0.41	0.0187	0.0009	0.0118		0.0179
10	67	0.918	3.80	0.725	0.56	0.0238	0.0011	0.0114	0.286	
10	68	0.842	3.89	0.736	0.88	0.0253	0.0008	0.0086		0.0336
10C	55	1.055	4.36	0.853	0.86	0.0175	0.0008	0.0104	0.216	
10C	56	1.081	4.98	0.782	1.62	0.0268	0.0012	0.0145		0.0340

a/ Fed 10% cotton linters.

TABLE 16

SUMMARY OF LESIONS IN DOGS FED NC FOR 12 MONTHS

Dosage (% in feed):	0		10		10C[a/]	
Dog No.:	51	52	67	68	55	56
Sex:	M	F	M	F	M	F
Lesions[b/]						
Lung						
Peribronchiolitis	1					
Perivasculitis	1					1
Pneumonia	1					
Liver						
Hemosiderosis				1		
Microfoci of mononuclear cells	1	1				1
Microgranulomas			1			
Small Intestine						
Roundworms (Ascarid)	1	1				
Inflammation		1				
Kidney						
Microcalculi in medulla	1	1	1	1	1	1
Ovary						
Mineralization		1				
Thyroid						
Parafollicular cell hyperplasia	1	2	1	2		1
Spleen						
Hemosiderosis	1	1	1	1	1	1
Inflammation		1				
Thymus						
Involution		1	1		2	1
Axillary Lymph Node						
Inflammation		1			1	1
Hemosiderosis		1	1	1	1	1
Mesenteric Lymph Node						
Inflammation		1				
Bone Marrow						
M/E ratio	1.3	1.3	1.2	1.3	1.2	1.3

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = minimal to mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present.

TABLE 17

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF DOGS FED NC FOR 24 MONTHS

Dose (% in feed)	Dog No.	Terminal Body Weight (kg)	Absolute Organ Weight (gm) Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	2	14.4	84.0	111.7	428.0	65.8	66.3	1.41	0.08	0.88		1.21
0	3	14.4	91.6	111.0	542.6	105.5	101.0	1.67	0.11	0.85	15.5	
0	4	13.6	84.3	102.6	506.8	66.1	77.1	1.81	0.11	1.01		0.98
0	5	15.0	91.2	97.7	420.4	75.5	127.0	1.46	0.10	1.00	15.5	
1	18	13.2	86.7	88.0	320.2	50.8	107.0	1.63	0.08	0.53		0.99
1	19	12.4	79.7	84.6	364.0	72.9	71.6	1.49	0.08	0.84	11.2	
1	20	11.7	70.6	86.4	438.6	58.0	48.6	1.55	0.08	0.76		1.14
1	21	17.4	96.2	135.7	591.8	69.4	149.7	1.93	0.09	1.13	19.5	
3	25	14.0	82.6	106.7	451.7	83.2	97.0	1.76	0.10	0.81	17.0	
3	26	9.7	85.9	83.2	373.0	51.9	72.4	1.89	0.08	0.74		1.19
3	27	13.0	81.9	106.4	379.0	74.5	71.6	1.57	0.08	1.10	17.2	
3	28	15.2	89.5	93.0	311.7	54.0	83.9	1.16	0.11	0.59		1.17
10	33	13.8	82.1	119.6	508.6	105.5	126.0	2.05	0.09	0.84	17.0	
10	34	11.7	79.0	86.1	431.7	58.3	164.8	2.06	0.09	0.93		1.94
10	35	14.8	90.5	102.8	376.0	74.1	110.0	1.28	0.06	0.72	14.7	
10	36	11.2	76.7	82.0	374.9	50.5	61.0	1.52	0.07	0.71		1.65
10C[a]	9	20.8	97.4	129.4	529.0	84.6	143.0	1.73	0.12	1.05	19.0	
10C	10	14.2	91.6	100.8	415.2	68.9	118.0	1.96	0.09	0.92		1.57
10C	11	14.8	90.6	114.1	403.2	86.9	93.6	1.01	0.08	0.77	12.1	
10C	12	11.2	67.3	77.6	299.0	51.9	25.4	1.68	0.09	0.94		0.89

Dose (% in feed)	Dog No.	Relative Organ Weight (gm/kg body weight) Brain	Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	2	5.83	7.76	29.7	4.57	4.60	0.098	0.006	0.061		0.084
0	3	4.98	6.77	33.0	6.43	6.16	0.102	0.007	0.052	0.95	
0	4	6.13	7.54	37.3	4.86	5.67	0.133	0.008	0.074		0.072
0	5	6.08	6.51	28.0	5.03	8.47	0.097	0.007	0.067	1.03	
1	18	6.56	6.67	24.3	3.85	8.11	0.124	0.006	0.040		0.071
1	19	6.43	6.77	29.4	5.88	5.77	0.120	0.007	0.068	0.90	
1	20	5.98	7.39	37.5	4.96	4.15	0.133	0.007	0.065		0.097
1	21	5.53	7.80	34.0	3.99	8.60	0.111	0.005	0.065	1.12	
3	25	5.90	7.62	32.3	5.94	6.93	0.126	0.007	0.058	1.21	
3	26	8.86	8.58	38.4	5.35	7.46	0.195	0.008	0.076		0.119
3	27	6.30	8.18	29.2	5.73	5.51	0.121	0.006	0.085	1.32	
3	28	5.89	6.12	20.5	3.55	5.52	0.076	0.007	0.039		0.077
10	33	5.94	8.62	36.9	7.64	9.13	0.149	0.007	0.061	1.23	
10	34	6.75	7.36	36.9	4.98	14.09	0.176	0.008	0.080		0.169
10	35	6.11	6.95	25.4	5.01	7.43	0.087	0.004	0.049	0.99	
10	36	6.85	7.32	33.5	4.51	5.45	0.135	0.006	0.063		0.159
10C	9	4.68	6.22	25.4	4.06	6.88	0.083	0.006	0.051	0.91	
10C	10	6.45	7.10	29.2	4.85	8.31	0.138	0.006	0.065		0.111
10C	11	6.12	7.71	27.2	5.87	6.32	0.068	0.005	0.052	0.82	
10C	12	6.01	6.93	26.7	4.63	2.27	0.150	0.008	0.084		0.080

Dose (% in feed)	Dog No.	Relative Organ Weight (gm/gm brain weight) Heart	Liver	Kidney	Spleen	Adrenal	Pituitary	Thyroid	Testis	Ovary
0	2	1.330	5.10	0.783	0.79	0.0168	0.0010	0.0105		0.0144
0	3	1.360	6.64	1.293	1.24	0.0205	0.0013	0.0104	0.190	
0	4	1.232	6.08	0.794	0.93	0.0217	0.0013	0.0121		0.0118
0	5	1.071	4.61	0.830	1.39	0.0160	0.0011	0.0110	0.170	
1	18	1.015	3.69	0.586	1.23	0.0188	0.0009	0.0061		0.0107
1	19	1.054	4.57	0.915	0.90	0.0187	0.0010	0.0105	0.141	
1	20	1.234	6.27	0.829	0.69	0.0221	0.0011	0.0109		0.0163
1	21	1.411	6.15	0.721	1.56	0.0201	0.0009	0.0117	0.203	
3	25	1.292	5.47	1.007	1.17	0.0213	0.0012	0.0098	0.206	
3	26	0.969	4.34	0.604	0.84	0.0220	0.0009	0.0086		0.0134
3	27	1.299	4.63	0.910	0.87	0.0192	0.0010	0.0134	0.210	
3	28	1.039	3.48	0.603	0.94	0.0130	0.0012	0.0066		0.0131
10	33	1.450	6.19	1.285	1.53	0.0250	0.0011	0.0102	0.207	
10	34	1.090	5.46	0.738	2.06	0.0261	0.0011	0.0118		0.0251
10	35	1.136	4.15	0.819	1.22	0.0141	0.0007	0.0080	0.162	
10	36	1.069	4.89	0.658	0.80	0.0197	0.0009	0.0093		0.0219
10C	9	1.339	5.43	0.869	1.47	0.0178	0.0012	0.0108	0.195	
10C	10	1.100	4.53	0.752	1.29	0.0214	0.0010	0.0100		0.0171
10C	11	1.259	4.45	0.959	1.03	0.0111	0.0009	0.0085	0.134	
10C	12	1.153	4.44	0.771	0.38	0.0250	0.0013	0.0140		0.0132

a/ Fed 10% cotton linters.

TABLE 18

SUMMARY OF LESIONS IN DOGS FED NC FOR 24 MONTHS

Dosage (% in feed):	0				1				3				10				10[a]			
Dog No.:	2	3	4	5	18	19	20	21	25	26	27	28	33	34	35	36	9	10	11	12
Sex:	F	M	F	M	F	M	F	M	M	F	M	F	M	F	M	F	M	F	M	F
Lesions[b]																				
Adrenal																				
Focal fatty change																		1		
Pituitary																				
Cyst formation				1		1	1					1	1		1		1		1	1
Thyroid and Parathyroid																				
Chronic lymphocytic thyroiditis								3										1	3	3
Follicle epithelium hyperplasia								1										1		
Lung																				
Lung worm infestation											1									
Pleural fibrosis	1	1		1			1	1					1	1	1				1	1
Muscular hypertrophy of bronchioles		1				1	1	1	1				1	1	1	1	1	1		
Granulomatous pneumonia		1		1				1		2		2		1	1		2			
Peribronchiolar with/without perivascular cuffs				1			1	1		1		1				1	2	1	1	
Liver																				
Granuloma			1				1				1	1								
Portal inflammation															1					1
Gall Bladder																				
Epithelial hyperplasia												1								1
Lymphoid hyperplasia			2		1															
Spleen																				
Extramedullary hematopoiesis	1											1								
Hemosiderosis																		+	1	1
Ectopic spleen									1					1	1					
Mesenteric Lymph Node																				
Eosinophilic granuloma (mesenteric)									1							1				1
Lymphoid hyperplasia											1									
Regional Lymph Node																				
Hemosiderosis		1		1		1		1				1	1			1				
Lymphoid hyperplasia					1						1							1		
Epididymis																				
Focal mononuclear cell infiltration		1																		
Prostate																				
Interstitial prostatitis						1					2						1			
Stomach																				
Lymphoid hyperplasia											1					1				
Intestine																				
Lymphoid hyperplasia	1																			
Parasitism	2																			
Foreign body					1															
Kidney																				
Renal calcinosis					1			1			1	1								1
Glomerular nephrosis								1					1							
Peripyelitis											1									
Esophagus																				
Focal mononuclear cell infiltration				1					1		1	1				1			1	
Tonsil																				
Focal tonsillitis			1								1	1	1	1	1		1			
Tongue																				
Glossitis						1			1	1								1		1
Eye																				
Hypertrophy of glans nictitans								2												
Salivary Gland																				
Focal mononuclear cell infiltration															1			1		
Mammary Gland																				
Focal mononuclear cell infiltration																				1
Bone Marrow Smear																				
M/E ratio	0.9	1.8	1.3	1.6	0.9	1.0	1.0	1.0	1.0	0.9	1.0	0.9	1.1	1.3	1.0	1.0	1.2	1.4	1.3	1.1

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; + = questionable; X = present.

IV. RAT STUDIES

TABLE OF CONTENTS

IV. RAT STUDIES

The following sections describe the results and interpretations of the rat studies.

A. Observations and Toxic Signs

Some adverse effects were observed, but these were seen scattered throughout all groups, and not related to NC feeding. Unscheduled deaths among males and females are summarized in Figures 4 and 5, respectively. In both sexes, none of the treated groups had more deaths than the normal controls, and none had fewer deaths than the cotton controls. Some data are omitted for the sake of clarity. Deaths among low (1% NC) and middle (3% NC) dose males were similar to those of high dose (10% NC) males. Deaths among low and middle dose females were intermediate between those control and high dose females.

Deaths came from a variety of causes. Laboratory data from rats dying at unscheduled times are given in Table 19. Some of the early deaths (Months 7 through 10) were symptomatic of pseudotuberculosis. Each rat typically had a rough coat, an arched back, rapid, noisy breathing, and weight loss, with the characteristic caseous lesions seen in the lung at necropsy. To minimize spread, animals with the signs were kept isolated until they recovered or became moribund.

The most common cause of death was tumors. The first variety observed (from Month 7) was subcutaneous tumors of various types, including mammary tumors. When these became ulcerated, we killed the rats to prevent suffering. In other cases, the tumor became so large that the rat appeared to be putting its metabolic effort in support of the tumor, rather than of its somatic tissues. The tumor would increase in size and the rest of the body waste away. An unusual variation was cotton control female No. 50-048. Occasionally, rats develop nevi (typically 2 mm x 2 mm x 10 mm long) on their tail. No. 50-048 had one about the middle of the tail which increased to 20 mm x 30 mm x 20 mm long and became ulcerated. She was isolated and observed, but the lesion neither healed nor regressed. The tail and lesion were amputated in Month 19, and the rat survived to the end of the study.

In the second year, we saw increasing numbers of pituitary chromophobe cell adenomas, a very common tumor in this strain of rats.[16/] These rats could be identified by their behavior. Most commonly, we saw unilateral ataxia which sometimes developed to paralysis. At times, the ataxia affected the entire body. Sometimes these rats, even if ataxic, were hyperexcitable, having exaggerated responses to stimuli. The more severe cases

were accompanied by severe weight losses--as much as 200 g in 2 weeks. If the loss was as much as 50 g/week for 2 or more weeks, the rat was considered moribund and killed.

Other deaths occurred from unusual or unknown causes. Typically, the only symptom observed was inanition with weight loss, inactivity, and rough coat. Often no gross lesions or specific abnormalities of laboratory data were found. However, some diagnoses were obvious, such as the malignant lymphoma in control male No. 51-101 (see laboratory data in Table 19). The most unusual was control male No. 51-107. In 4 weeks, his weight dropped from 730 g to 548 g. He showed typical, nonspecific inanition, and was killed in Month 19. At necropsy, he looked like a case of adiposogenital dystrophy (Froehlich's Syndrome) with a large amount of abdominal fat (despite weight loss) and very small testes (0.74 g).

B. Body Weight

Average body weights of rats fed NC are shown in Figure 6. Some data are omitted to improve clarity. Control rats had rapid weight gains from the start of the study. The rate of gain decreased as the rats matured, reaching plateaus of 775 g in males and 375 g in females after about 1 year. There were additional weight gains later due to obesity, and fluctuations due to tumor weights (increasing) and to inanition of the rats (decreasing).

Rats fed the low or middle dose were substantially the same as control rats. However, high dose rats and cotton control rats failed to gain weight or even lost weight in the first week. Thereafter, they gained more slowly, reaching plateaus of 575 to 600 g in males and 325 to 350 g in females. Fluctuations and later weight gains were similar to those in controls, causing a convergence of average body weights in the last months of the study.

C. Feed Consumption

The average feed consumption data for males and females are shown in Figures 7 and 8, respectively. Averages are listed in Table 20. These averages are labelled "apparent" because the high dose and cotton control rats had visible scattering of feed and fiber around their cages, which accounted for part of the loss of weight in the feeders (the measured parameter).

All dosed rats had a doserelated increase in apparent feed consumption. This is consistent with the fact that NC acts as nonnutritive bulk. As shown in the figures, there were major variations in consumption from month to month. The only timerelated trend was a decrease in feed scattering and, therefore, in apparent consumption, in highdose and cotton control rats. In both sexes, the rats in these two dosage groups consumed less feed in the second year than in the first.

D. Laboratory Data

Baseline values for hematology data of males and females given NC are in Tables 21 and 22, respectively. The values for all groups are normal, with only insignificant differences between groups.

Laboratory data after 6, 12, 18 and 24 months of feeding are shown in Tables 23 through 30. There are occasional differences between the groups, but these are usually minor and always inconsistent. The large differences are caused by aberrant individuals. For instance, the atypical cells in all the males tested after 24 months feeding (Table 29) were the 67% immature lymphocytes in control male No. 51-101. If his data were omitted, the control values for the neutrophils and lymphocytes of the controls averaged 34 and 65%, respectively, practically identical to those of the other groups.

The reversal study after 12 months' feeding was omitted because no adverse effects were observed. Laboratory data from rats fed 24 months and allowed to recover for 1 month are shown in Tables 31 and 32. All control males had died before the end of the study, so comparisons for the males were made to the cotton control. As described above, only minor, unimportant variations were noted.

E. Pathology

Data are presented on 21 male and 30 female control rats, 27 male and 35 female high dose rats and 32 male and 35 female cotton control rats. Missing rats include those not necropsied (recovery after 12 months feeding) and those lost to decomposition, cannibalism, etc.

1. Feeding for 12 Months

Absolute and relative organ weights of rats fed NC for 12 months are listed in Table 33. There are a few statistically significant differences. These differences, especially those in spleen weight, represent normal variation.

Tissue lesions in male and female rats fed NC for 12 months are shown in Tables 34 and 35, respectively. Chronic murine pneumonia was found in all control and all treated rats. A variety of other lesions was found

in these rats. There was no relationship between NC feeding and any of the lesions, in incidence or severity of the lesions. Therefore, the low and middle dose rats' slides were not read and the recovery necropsy after 1 month's recovery was omitted.

2. Feeding for 24 Months

Absolute and relative organ weights of rats fed NC for 24 months are shown in Table 36. There were no statistically significant differences between treatment groups.

A large number of lesions were found in various tissues of rats fed NC for 24 months, as shown in Tables 37 through 42. Some lesions, such as chronic murine pneumonia, were found in most rats. Other lesions were found more sporadically. The variety and incidence are typical of geriatric rats. No lesion was related, in incidence or severity, to the NC dose given.

Lesions from rats fed NC for 24 months and allowed to recover for 1 month are shown in Tables 43 and 44. The results were the same as seen in rats not allowed to recover. No NC-related lesions were seen. Lesions from rats dying at unscheduled times, including the only control male surviving to begin the recovery study, are listed in Tables 45 through 53. As described above, there were no NC-related lesions.

The incidence of tumors in the preceding tables has been listed in Table 54. As reported in the literature,[16] the only common tumors were the pituitary chromophobe cell adenoma and various mammary tumors, including fibroadenomas, adenomas, and adenocarcinomas/carcinomas. There was a scattered incidence of many other tumor types, but no relationship to NC feeding.

F. Three-Generation Reproduction Study

As indicated in Table 55, the mean body weights at the time of first matings for males of all parental generations, given diets containing 10% of either NC or cotton linters, were significantly reduced when compared to males given normal control diet. These means for all parental generations given 10% cotton linters were also significantly reduced when compared to males given 10% NC. In the females, only the mean body weights at the time of first matings for those given diets containing 10% cotton linters were significantly reduced when compared to females given control diet.

No indications that the treatments adversely affected the fertility of the males or females were apparent in the mating ratio, pregnancy ratio, or the ratios of fertile to mated males or females. For the F_0 generation, most of these parameters suggest that either 10% NC or cotton linters in the diet increased the fertility of rats given these treatments. However, this effect appears to be caused by the decreased fertility of the control F_0 females. The fertility for F_0 females was similar for groups given 1 and 3% NC. This decreased fertility in the F_0 groups was associated with both an age at time of first mating and body weight greater than that expected to give optimal reproductive performance.

The data for the various litters are detailed in Table 56. No treatment effects were apparent on litter size, liveborn index, birth weight, viability index, or the ratio of males to total offspring. Significant reductions in the lactation index and the weight of pups at weaning occurred with both the 10% NC and cotton linter groups. Reductions in these parameters were observed chiefly with the F_{1b} through F_{2b} litters. Similar reductions were not observed with the subsequent litters.

As in the chronic toxicity, rats given diets containing 10% fiber ate more than the other rats, presumably to compensate for the inert fiber content of the feed. Mean feed intake values for the control, 10% NC, and 10% cotton linter groups were 81, 103 and 118 g/day, respectively, during lactation of the F_{2b}; and 62, 74 and 72 g/day, respectively, during lactation of the F_{3b}. Feed consumption measurements on other groups were not made.

This study suggests that NC alone at dose levels as high as 10% in the diet does not affect reproduction, but that the increased inert bulk of 10% fibers (NC or linters) can cause some adverse effects on reproduction.

G. Mutagenesis Study

The results of the chromosome analysis of bone marrow and kidney cultures from rats fed NC for 24 months are shown in Tables 57 and 58. There were no increases in chromosome abnormalities.

H. Discussion and Conclusions

There is no evidence of any adverse effect associated with the feeding of nitrocellulose to rats. Rats fed the high dose (10%) or an equal amount of the inert cotton linters did have some effects when compared with rats fed less (or no) NC. These effects, which were often more obvious in the cotton control rats, are presumably due to presence of a relatively large amount of inert bulk in the diet.

Rats fed the high dose or cotton control diets had a somewhat better survival rate. This result is probably associated with their somewhat lower body weight, which was apparently due to decreased obesity, rather than decreased lean body mass. To cite the extreme case, one low dose male (No. 42-146) weighed as much as 1,042 g before he was found dead and decomposed in week 102, but no high dose or cotton control male ever weighed as much as 850 g. Weight differences among females were less, and were confounded, especially in the later months, by the presence of tumors, often large.

There was a dose-relationship in feed consumption, as would be expected from addition of an inert bulk ingredient to the diet. However, the relationship was not proportional to the added bulk. Addition of 10% fiber (NC or linters) caused a qualitative behavioral change. The rats would waste large quantities of the diet, apparently attempting to separate fiber from feed.

Other than biological variation, no effects were seen in the clinical chemistry, gross pathology, and histopathology. If NC is denitrated in the gut, then the amount of nitrate/nitrite formed is presumably within the ability of the body to detoxify. The special toxicity tests, including immunoglobin E assay, cytogenetic analysis, three-generation reproduction, also showed no adverse effects of the NC, although the fiber consumption and consequent lower body weight had inconsistent adverse effects in the reproduction study.

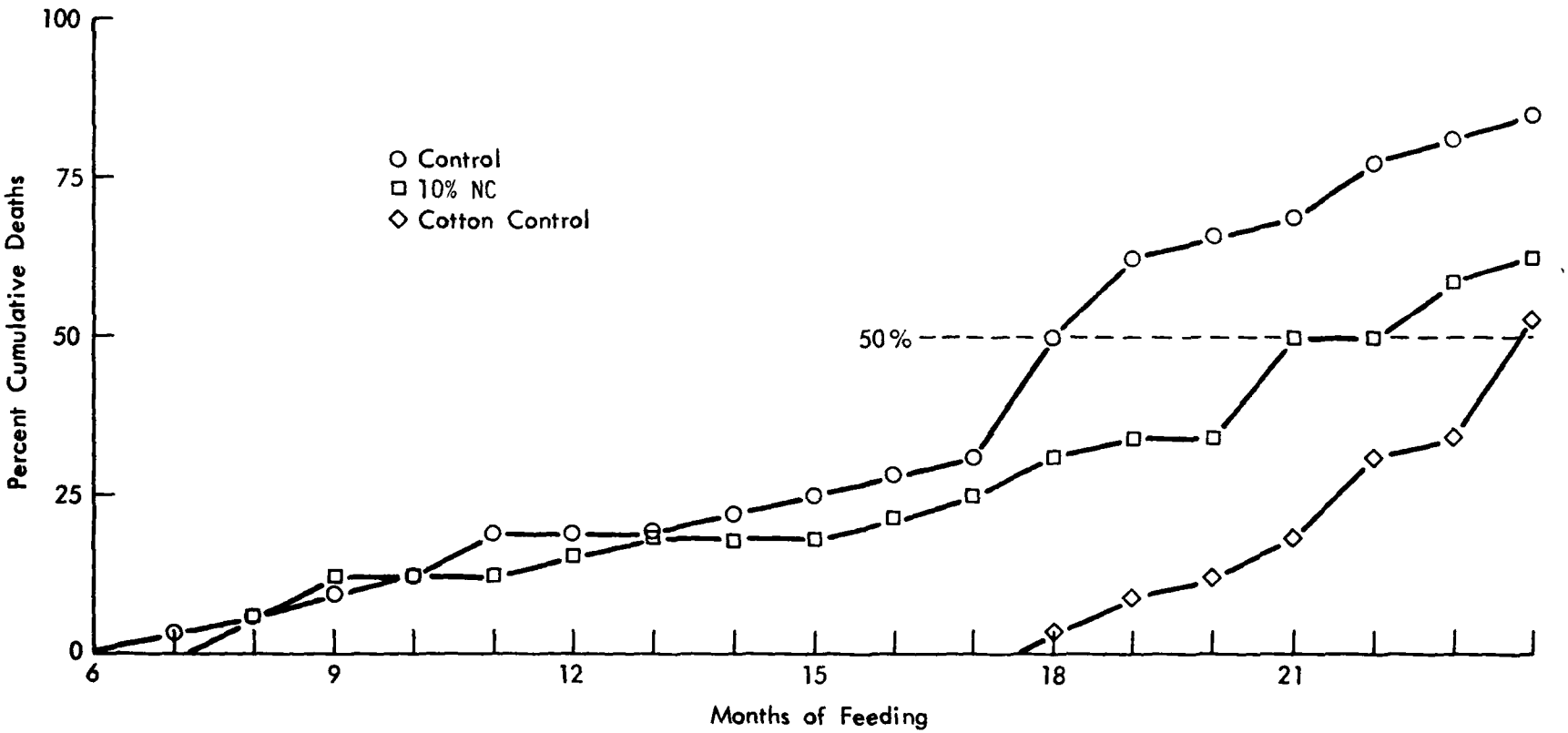

Figure 4 - Cumulative Unscheduled Deaths Among Male Rats Fed NC

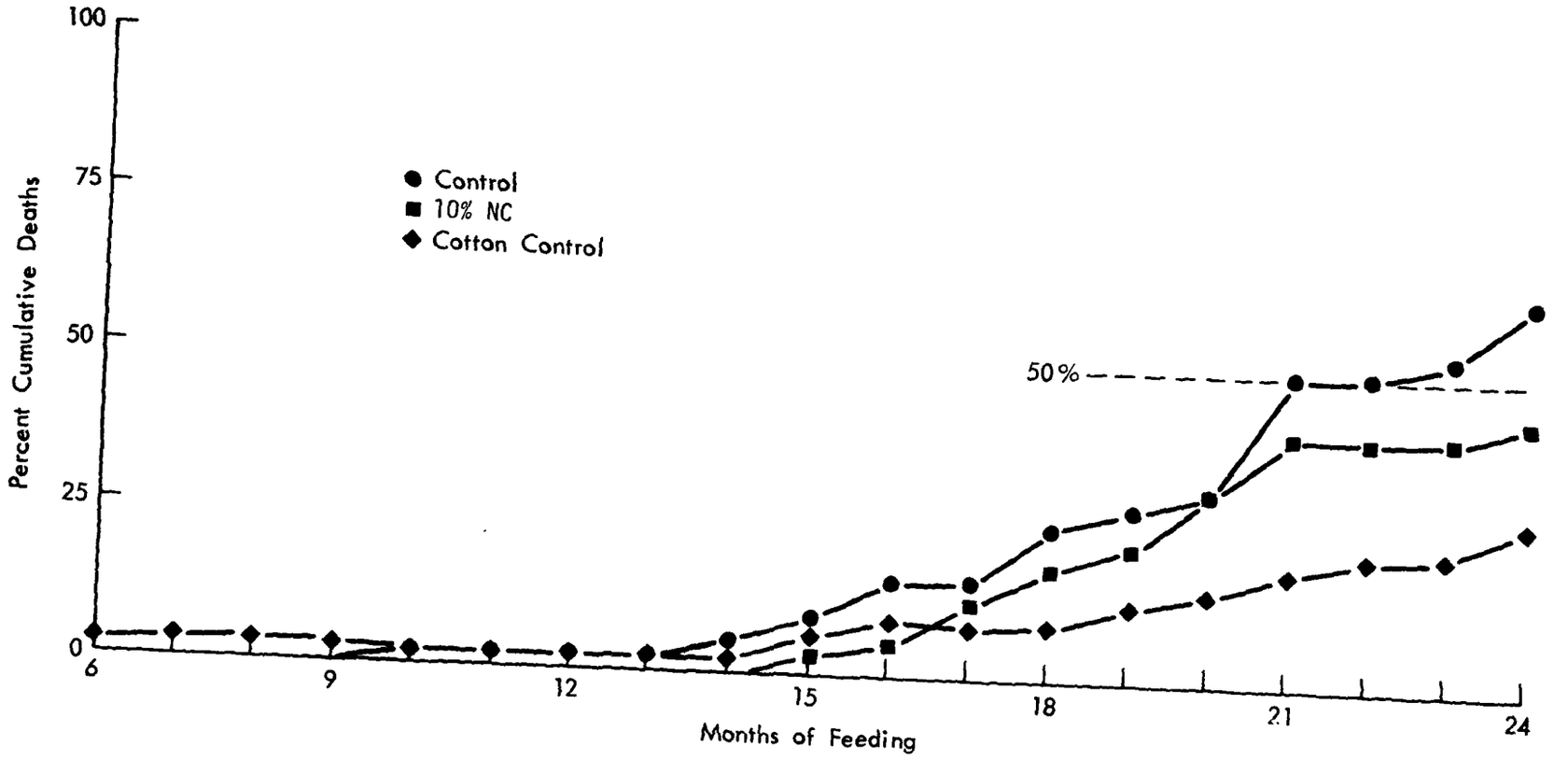

Figure 5 - Cumulative Unscheduled Deaths Among Female Rats Fed NC

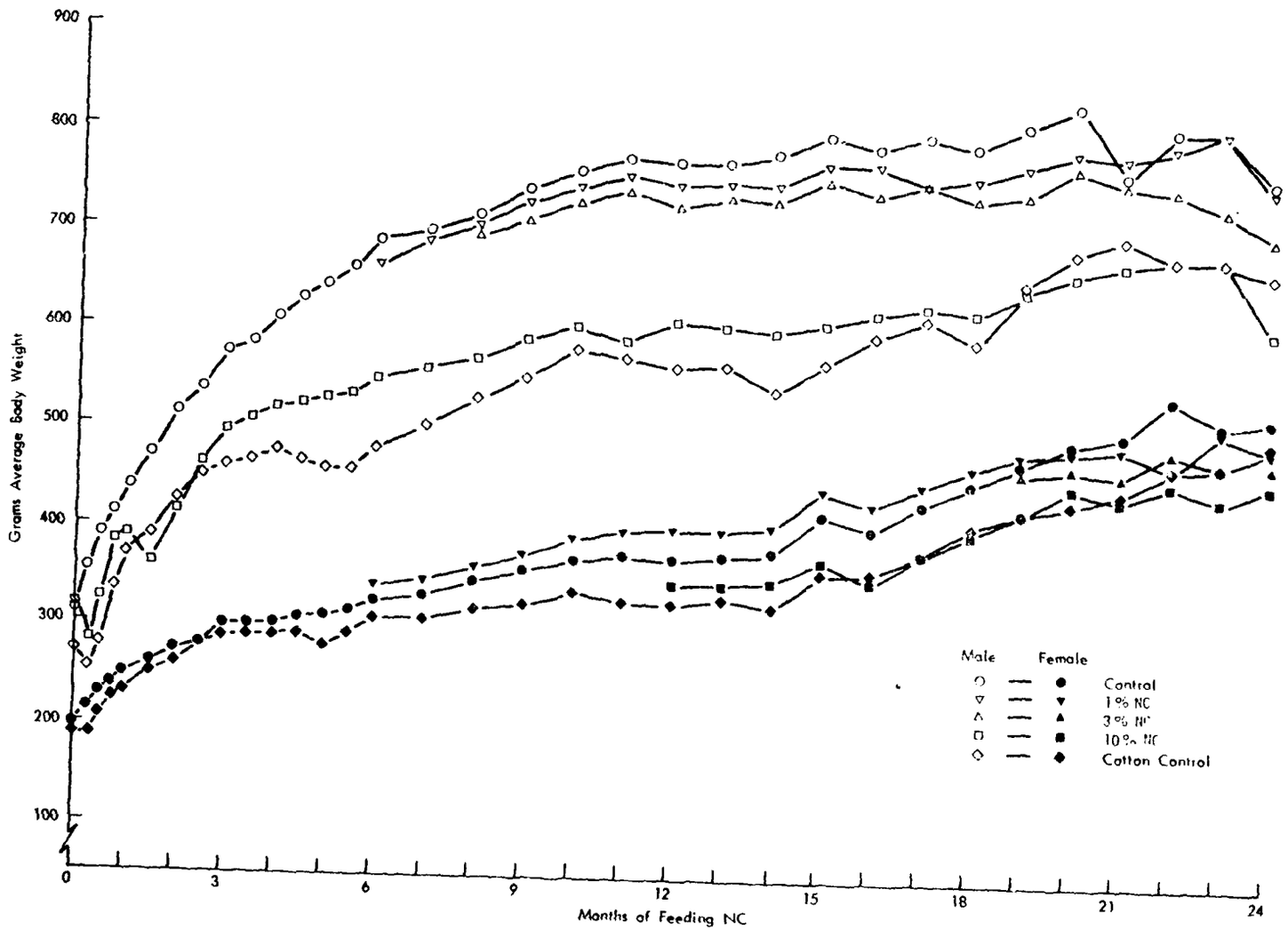

Figure 6 - Average Body Weights of Rat Fed NC .

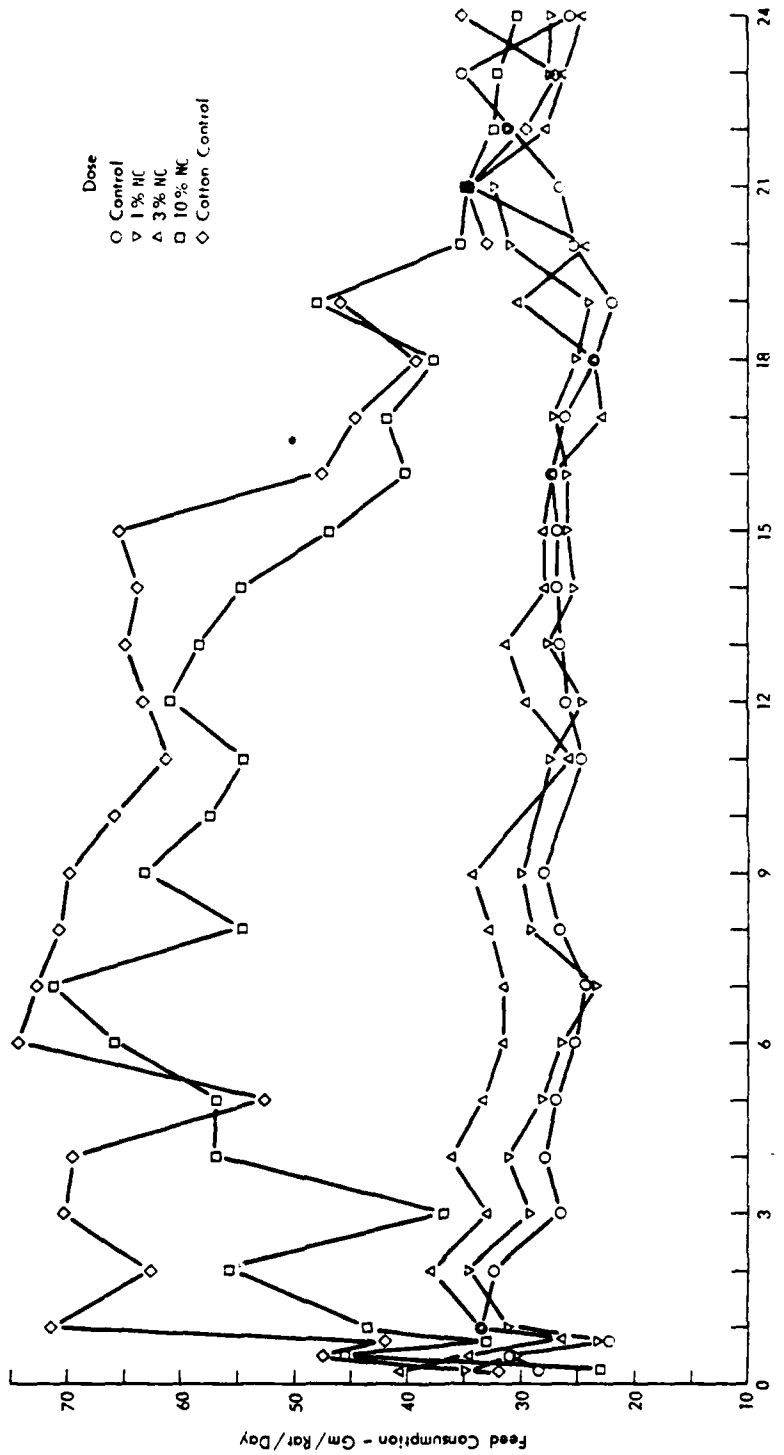

Figure 7 - Feed Consumption of Male Rats Fed NC

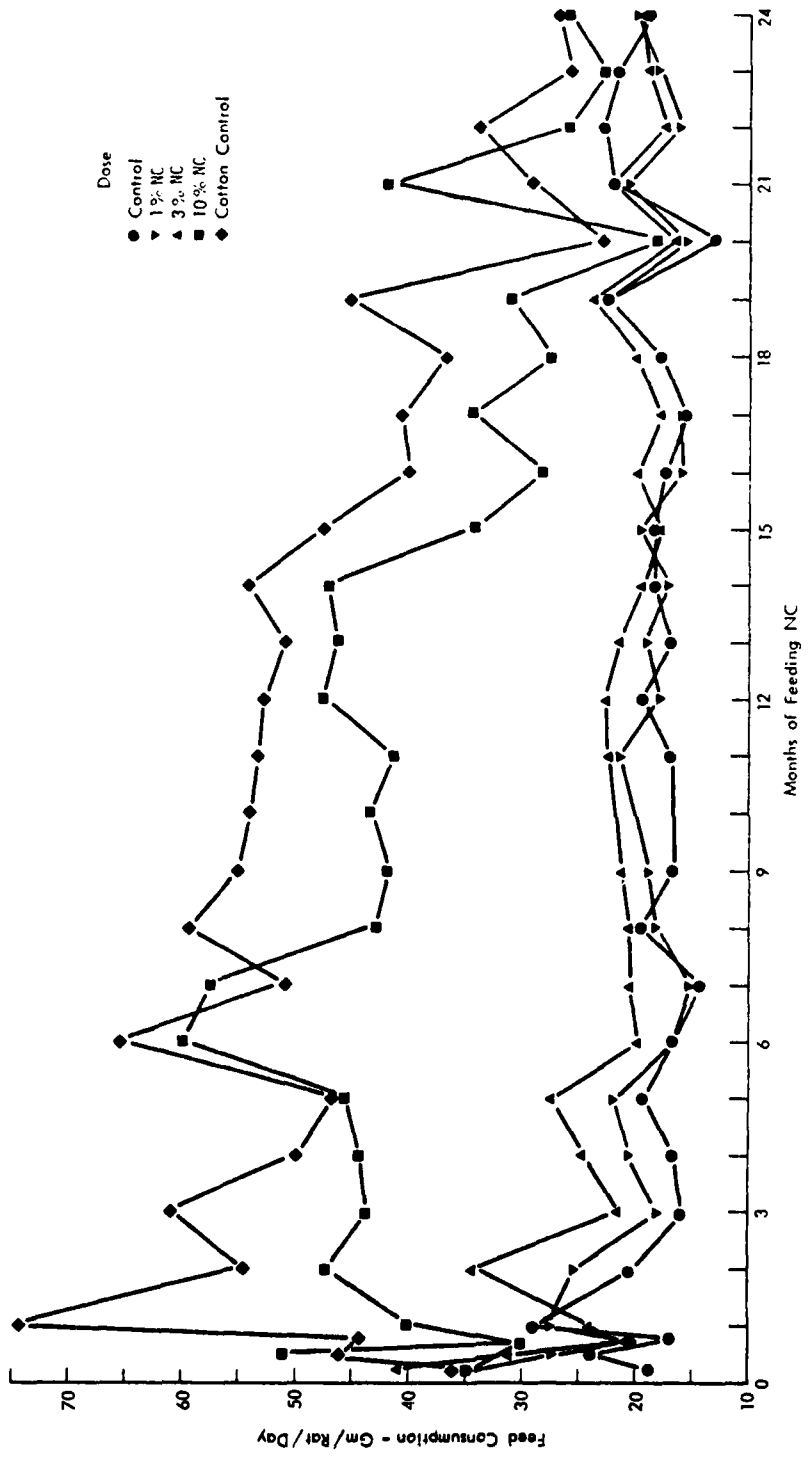

Figure 8 - Feed Consumption of Female Rats Fed NC

TABLE 19

LABORATORY DATA OF RATS FED NC AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	3	3	0	0	3	10[a/]	0	1	10	10
Rat No:	53-262	53-268	41-124	51-118	53-165	50-049	51-218	42-241	54-293	44-287
Week of Death:	53	58	58	61	61	61	61	63	63	66
Erythrocytes, x 10^6/mm^3	4.44	4.64	7.96	6.87	9.34	5.82	6.67	5.24	7.16	1.61
Heinz bodies, %	0.00	0.00	0.00	-	0.00	0.00	0.00	-	0.00	4.81
Reticulocytes, %	2.07	1.87	1.81	0.87	3.38	1.42	2.49	2.27	1.55	2.70
Hematocrit, vol %	35	33	48	37	62	39	40	39	44	11
Hemoglobin, g %	11.9	11.4	15.8	13.7	19.8	12.8	13.2	12.5	14.3	3.6
Methemoglobin, %	0.0	0.0	0.0	-	0.4	0.0	0.0	-	0.0	0.0
MCV, cubic microns	78.8	71.1	60.3	53.9	66.4	67.0	60.0	74.4	61.5	68.3
MCHB, picograms	26.8	24.6	19.8	19.9	21.2	22.0	19.8	23.9	20.0	22.4
MCHBC, g %	34.0	34.5	23.9	37.0	31.9	32.8	33.0	32.1	32.5	32.7
Platelets, x 10^5/mm^3	7.40	6.20	2.90	4.00	3.20	3.95	3.50	2.40	2.95	3.20
Leucocytes, x 10^3/mm^3	13.4	4.8	5.7	11.7	11.3	4.5	4.6	48.3	52.0	17.6
Neutrophils, %	40	23	29	60	35	22	41	88	70	29
Lymphocytes, %	57	74	65	36	64	76	57	11	27	70
Bands, %	1	0	1	1	0	0	0	0	0	1
Eosinophils, %	0	3	2	3	0	2	0	0	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0
Monocytes, %	2	0	2	0	1	0	2	1	3	0
Atypical, %	0	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	5	0	0	0	4	0	0
Glucose, mg %	174	140	178	117	180	133	181	81	155	218
SGOT, IU/ℓ	34	46	183	55	168	74	46	294	148	96
SGPT, IU/ℓ	18	24	49	37	80	28	21	43	31	18
Alkaline phosphatase, IU/	35	14	44	26	44	28	22	414	467	106
BUN, mg %	21	17	14	170	20	16	18	114	17	24
IgE, IU/ℓ	1,550	1,000	850	-	950	200	550	900	900	950

(Continued)

TABLE 19 (Continued)

Dose (% in feed):	0	0	10C[a/]	3	3	3	10	0	1
Rat No.:	51-206	51-102	40-203	43-268	53-274	53-272	54-193	41-227	52-137
Week of Death:	67	67	68	68	68	71	71	77	77
Erythrocytes, x 10^6/mm^3	5.58	7.14	2.84	5.44	5.43	5.36	3.62	6.03	4.75
Heinz bodies, %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reticulocytes, %	0.54	1.85	16.06	1.48	1.28	4.52	2.11	1.33	1.43
Hematocrit, vol %	41	47	29	37	40	36	24	38	33
Hemoglobin, g %	13.4	15.1	8.2	12.2	13.3	11.7	8.4	12.9	11.7
Methemoglobin, %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.7
MCV, cubic microns	73.5	65.8	102.1	68.0	73.7	67.2	66.3	63.0	69.5
MCHB, picograms	24.0	21.1	28.9	22.4	24.5	21.8	23.2	21.4	24.6
MCHBC, g %	32.7	32.1	28.3	33.0	33.3	32.5	35.0	33.9	35.5
Platelets, x 10^5/mm^3	6.15	7.45	4.80	4.10	3.75	4.00	5.55	4.45	1.65
Leucocytes, x 10^3/mm^3	4.3	7.1	8.1	8.8	6.4	14.0	25.1	8.9	10.5
Neutrophils, %	32	43	27	56	37	34	33	38	47
Lymphocytes, %	66	52	71	41	60	66	64	59	51
Bands, %	0	0	0	0	1	0	0	0	0
Eosinophils, %	2	1	0	3	1	0	2	1	1
Basophils, %	0	0	0	0	0	0	0	0	0
Monocytes, %	0	4	2	0	1	0	1	2	1
Atypical, %	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0	0	0
Glucose, mg	85	166	146	139	113	164	178	91	95
SGOT, IU/ℓ	152	55	90	59	99	127	46	86	77
SGPT, IU/ℓ	108	31	28	31	46	77	31	24	28
Alkaline phosphatase, IU/ℓ	9	53	46	40	36	44	51	34	19
BUN, mg %	11	14	17	17	17	16	20	14	16
IgE, IU/ℓ	1,100	1,300	1,575	2,175	1,550	1,700	1,325	550	1,150

(Continued)

TABLE 19 (Continued)

Dose (% in feed):	10	1	1	3	3	3	10	10[a/]	0
Rat No.:	54-277	52-248	52-249	53-267	43-264	43-267	54-298	50-014	51-107
Week of Death:	77	82	82	82	82	82	82	82	83
Erythorocytes, x $10^6/mm^3$	4.81	3.24	2.05	2.62	5.40	2.85	3.22	5.19	5.60
Heinz bodies, %	0.00	-	-	-	-	-	0.00	-	0.00
Reticulocytes, %	7.13	0.72	0.90	2.20	2.10	1.27	0.93	0.69	0.65
Hematocrit, vol %	31	42	39	41	44	38	40	50	37
Hemoglobin, g %	10.7	12.6	12.7	13.0	14.1	12.2	12.6	16.4	12.9
Methemoglobin, %	0.0	-	-	-	-	-	0.0	-	0.0
MCV, cubic microns	64.4	129.6	190.2	156.5	81.5	133.3	124.2	96.3	66.1
MCHB, picograms	22.2	38.9	62.0	49.6	26.1	42.8	39.1	31.6	23.0
MCHBC, g %	34.5	30.0	32.6	31.7	32.0	32.1	31.5	32.8	34.9
Platelets, x $10^5/mm^3$	2.95	2.25	1.50	2.15	4.25	1.50	2.50	2.60	4.20
Leucocytes, x $10^3/mm^3$	8.9	7.9	4.7	5.5	4.4	6.5	9.5	7.9	8.2
Neutrophils, %	22	23	9	24	31	19	23	56	22
Lymphocytes, %	77	72	89	73	69	79	76	44	75
Bands, %	0	0	0	0	0	0	0	0	1
Eosinophils, %	0	3	2	2	0	0	0	0	2
Basophils, %	0	0	0	0	0	0	0	0	0
Monocytes, %	1	2	0	1	0	2	1	0	0
Atypical, %	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0	0	0
Glucose, mg %	115	128	166	103	139	154	117	113	103
SGOT, IU/ℓ	68	71	77	105	93	80	108	124	52
SGPT, IU/ℓ	18	18	34	28	28	43	34	62	24
Alkaline phosphatase, IU/ℓ	24	60	57	19	41	44	28	14	40
BUN, mg %	14	15	16	11	16	17	14	9	17
IgE, IU/ℓ	1,300	-	-	-	-	-	1,050	2,050	-

(Continued)

TABLE 19 (Continued)

Dose (% in feed):	10[a/]	0	3	3	10	10	10[a/]	10[a/]	10[a/]	10[a/]
Rat No.:	50-007	51-114	53-161	53-263	54-281	54-288	50-011	40-103	50-013	50-015
Week of Death:	83	84	84	84	84	84	93	93	96	101
Erythrocytes, x 10^6/mm^3	4.65	5.25	4.35	3.37	3.16	4.29	6.89	6.53	5.84	5.10
Heinz bodies, %	-	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00
Reticulocytes, %	3.40	1.30	1.17	0.37	0.25	1.19	2.26	1.06	1.27	0.91
Hematocrit, vol %	27	39	39	42	35	39	41	39	36	32
Hemoglobin, g %	8.0	13.8	13.1	12.9	12.1	13.3	13.2	12.7	11.9	10.4
Methemoglobin, %	-	-	-	-	4.1	0.0	0.0	0.0	0.0	0.0
MCV, cubic microns	58.1	74.3	89.7	124.6	110.8	90.9	59.5	59.7	61.6	62.7
MCHB, picograms	17.2	26.1	30.1	38.3	38.0	31.0	19.2	19.4	20.4	20.4
MCHBC, g %	29.6	35.4	33.6	30.7	34.5	34.1	32.2	32.6	33.1	32.5
Platelets, x 10^5/mm^3	5.45	6.85	4.75	3.30	4.25	3.50	6.50	3.65	8.00	5.35
Leucocytes, x 10^3/mm^3	6.4	11.6	7.4	5.1	4.4	4.9	9.8	13.9	6.2	21.4
Neutrophils, %	27	46	28	22	33	23	25	53	23	73
Lymphocytes, %	71	48	70	73	63	74	69	41	75	27
Bands, %	0	0	0	0	0	0	0	1	0	0
Eosinophils, %	0	2	2	1	1	3	3	1	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0
Monocytes, %	2	4	0	4	3	0	3	4	2	0
Atypical, %	0	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0	3	0	0
Glucose, mg %	100	64	62	80	85	154	139	102	74	98
SGOT, IU/ℓ	133	99	99	93	80	142	68	220	74	71
SGPT, IU/ℓ	31	21	24	28	24	80	34	43	24	18
Alkaline phosphatase, IU/ℓ	33	39	49	12	14	40	51	55	10	122
BUN, mg %	13	13	14	11	16	15	20	16	17	11
IgE, IU/ℓ	-	-	-	-	-	-	850	2,250	850	500

(Continued)

TABLE 19 (Concluded)

Dose (% in feed):	3	3	3	10C[a/]	0	1	0	10C[a/]
Rat No.:	52-235	52-227	52-236	40-104	51-217	42-247	51-101	54-282
Week of Death:	101	101	101	104	104	104	107[b/]	108[c/]
Erythrocytes, x 10^6/mm^3	3.30	3.49	5.87	4.75	6.81	3.90	4.15	3.42
Heinz bodies, %	-	-	-	0.00	0.00	-	0.00	0.00
Reticulocytes, %	2.91	2.50	1.78	9.78	1.33	1.56	5.94	0.65
Hematocrit, vol %	24	27	38	33	44	30	27	35
Hemoglobin, g %	8.6	8.3	12.4	11.4	15.3	9.7	8.7	11.3
Methemoglobin, %	-	-	-	0.0	0.0	-	0.0	0.0
MCV, cubic microns	72.7	77.4	64.7	69.5	64.6	76.9	65.1	102.3
MCHB, picograms	26.1	23.8	21.1	24.0	22.5	24.9	21.0	33.0
MCHBC, g %	35.8	30.7	32.6	34.5	34.8	32.3	32.2	32.3
Platelets, x 10^5/mm^3	1.00	4.50	4.50	5.40	6.85	4.65	1.80	5.00
Leucocytes, x 10^3/mm^3	7.1	12.2	7.8	8.4	7.9	11.0	308	5.6
Neutrophils, %	79	55	59	37	49	33	3	55
Lymphocytes, %	21	42	38	61	48	64	8	42
Bands, %	0	0	0	0	0	0	0	0
Eosinophils, %	0	0	1	1	1	1	0	1
Basophils, %	0	0	0	0	0	0	0	0
Monocytes, %	0	3	2	0	2	2	0	2
Atypical, %	0	0	0	0	0	0	89[d/]	0
Nucleated erythrocytes, %	99	138	120	140	123	99	44	76
Glucose, mg %	65	49	49	65	59	46	223	68
SGOT, IU/	390	15	24	77	21	21	46	17
SGPT, IU/	55	29	50	74	17	120	76	28
BUN, mg %	68	14	18	18	17	13	49	15
IgE, IU/	-	-	-	< 500	< 490	-	2,600	1,450

a/ Fed 10% linters.
b/ Died in Week 3 of recovery study.
c/ Died in Week 4 of recovery study.
d/ Eighty-six prolymphocytes and three lymphoblasts; marked anisocytosis, many microcytes.

TABLE 20

APPARENT FEED CONSUMPTION OF RATS FED NC FOR 24 MONTHS

Dose (% in Feed)	Apparent Feed Consumption (g/rat/day)					
	First Year		Second Year		Both Years	
	Males	Females	Males	Females	Males	Females
0	27.21 ± 0.65[b/]	18.12 ± 0.68	27.10 ± 0.97[c/]	18.99 ± 0.84	27.15 ± 0.58[d/]	18.57 ± 0.54
1	28.70 ± 0.93	20.25 ± 1.10	27.71 ± 0.78	18.26 ± 0.60	28.19 ± 0.60	19.21 ± 0.65
3	32.79 ± 0.96	24.09 ± 1.37	27.53 ± 0.97	19.29 ± 0.69	30.05 ± 0.87	21.59 ± 0.89
10	55.92 ± 3.28[e/]	46.60 ± 1.97	41.27 ± 2.66[e,f/]	31.92 ± 2.64[e,f/]	48.27 ± 2.57	38.94 ± 2.26
10C[a/]	65.25 ± 2.55[e/]	54.57 ± 1.64[e/]	43.92 ± 3.61[e,f/]	37.98 ± 2.96[e,f/]	54.12 ± 3.16[e/]	45.91 ± 2.45

a/ Fed 10% linters.

b/ Mean ± standard error of 11 measurements; the first month is the average of four measurements.

c/ Mean ± standard error of 12 measurements.

d/ Mean ± standard error of 23 measurements; the first month is the average of four measurements.

e/ Significantly different from control by Dunnett's multiple comparison procedure.

f/ Significantly different from first year data by Student's "t" test.

TABLE 21

LABORATORY DATA OF MALE RATS BEFORE FEEDING OF NITROCELLULOSE

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10C[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.32 ± .20	6.08 ± .14	6.30 ± .21	6.13 ± .25	6.20 ± .42
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	6.15 ± .40	6.45 ± .64	6.91 ± .16	5.18 ± .73	4.53 ± 1.06
HEMATOCRIT, VOL. %	47.5 ± 1.2	46.3 ± .3	45.8 ± 1.5	47.8 ± 2.3	45.5 ± 3.8
HEMOGLOBIN, GM. %	15.0 ± .4	14.6 ± .1	14.8 ± .5	14.8 ± .7	14.7 ± 1.0
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	75.2 ± .8	76.1 ± 1.5	72.6 ± 1.2	77.9 ± .9	73.0 ± 1.8
MCHB, MICRO MICROGMS.	23.7 ± .4	24.1 ± .5	23.4 ± .2	24.1 ± .2	23.7 ± .4
MCHBC, GM %	31.6 ± .2	31.6 ± .2	32.3 ± .5	31.0 ± .2	32.6 ± .9
PLATELETS ($X10^5$ /MM^3)	6.7 ± 1.1	6.5 ± .6	5.7 ± .1	7.2 ± .4	5.8 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	18.0 ± 1.9	15.4 ± 1.2	17.4 ± 3.0	17.9 ± 1.7	20.0 ± .9
NEUTROPHILS, %	11.0 ± 2.1	16.0 ± 1.8	14.3 ± .5	15.5 ± 2.5	15.3 ± 1.7
LYMPHOCYTES, %	87.8 ± 2.6	83.3 ± 1.9	85.0 ± .8	84.5 ± 2.5	84.3 ± 2.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.3 ± .3	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .6	.8 ± .5	.5 ± .5	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	.5 ± .5	.3 ± .3	.3 ± .3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 22

LABORATORY DATA OF FEMALE RATS BEFORE FEEDING OF NITROCELLULOSE

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.93 ± .10	5.84 ± .22	5.88 ± .05	5.85 ± .10	5.96 ± .25
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	4.42 ± 1.18	7.04 ± .70	5.45 ± .98	5.90 ± .79	6.04 ± .08
HEMATOCRIT, VOL. %	48.3 ± 1.4	44.0 ± 1.1	44.3 ± .9	42.8 ± .9[b/]	47.3 ± 1.3
HEMOGLOBIN, GM. %	15.2 ± .5	13.9 ± .5	13.8 ± .2	13.8 ± .3	14.4 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	81.3 ± 1.7	75.4 ± 1.2	75.3 ± 1.8	73.1 ± 2.1[b/]	79.4 ± 1.2
MCHB, MICRO MICROGMS.	25.6 ± .6	23.9 ± .2[b/]	23.5 ± .4[b/]	23.5 ± .2[b/]	24.2 ± .5
MCHBC, GM %	31.5 ± .1	31.7 ± .3	31.3 ± .2	32.3 ± 1.0	30.5 ± .6
PLATELETS ($X10^5$ /MM^3)	6.3 ± .6	7.1 ± .7	8.4 ± .4	8.1 ± .5	6.9 ± .8
LEUKOCYTES ($X10^3$ /MM^3)	19.0 ± 2.4	17.8 ± 1.8	18.6 ± 1.1	15.7 ± .9	17.5 ± 1.3
NEUTROPHILS, %	8.3 ± 2.1	5.3 ± .3	12.5 ± 1.3	11.0 ± 2.2	6.8 ± .6
LYMPHOCYTES, %	90.8 ± 2.6	93.3 ± .6	85.0 ± 2.3	87.8 ± 2.4	92.5 ± .6
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.5 ± .5	1.5 ± .5	.5 ± .5	.8 ± .3	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	0.0 ± 0.0	2.0 ± 1.1	.5 ± .3	.8 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	.5 ± .5	.3 ± .3	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNCAN'S MULTIPLE COMPARISONS PROCEDURE).

TABLE 23

LABORATORY DATA OF MALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 6 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0.00 (C, 4)	1.00 (T, 4)	3.00 (T, 4)	10.00 (T, 4)	10C[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.56 ± .22	8.10 ± .13	7.83 ± .22	7.37 ± .42	7.27 ± .35
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.80 ± .04	.83 ± .20	.63 ± .14	.30 ± .04[b/]	.61 ± .11
HEMATOCRIT, VOL. %	49.8 ± .5	50.3 ± 1.4	48.5 ± 1.2	51.3 ± .9	50.0 ± 2.2
HEMOGLOBIN, GM. %	16.6 ± .1	16.8 ± .3	16.5 ± .4	16.9 ± .3	16.0 ± .5
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	66.0 ± 2.0	62.0 ± .8	61.9 ± .3	70.1 ± 3.0	68.9 ± 1.4
MCHB, MICRO MICROGMS.	22.0 ± .6	20.7 ± .1	21.1 ± .2	23.1 ± .9	22.0 ± .4
MCHBC, GM %	33.4 ± .2	33.4 ± .4	34.0 ± .2	33.0 ± .1	32.0 ± .6
PLATELETS ($X10^5$ /MM^3)	6.3 ± .5	6.2 ± 1.0	6.9 ± 1.2	5.7 ± .3	6.5 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	17.2 ± .3	14.6 ± 1.5	15.8 ± 1.4	12.1 ± 2.3	18.3 ± 2.6
NEUTROPHILS, %	8.8 ± 1.4	10.8 ± 1.9	10.8 ± 3.1	14.0 ± 3.3	18.0 ± 4.8
LYMPHOCYTES, %	89.5 ± 1.3	88.0 ± 2.2	87.8 ± 3.4	83.8 ± 3.7	80.0 ± 5.7
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.3 ± .5	.5 ± .5	1.3 ± .5	2.3 ± .5	1.5 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.8 ± .5	.3 ± .3	0.0 ± 0.0	.5 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 24

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 6 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0.00 (C, 4)	1.00 (T, 4)	3.00 (T, 4)	10.00 (T, 4)	10C[a/] (C, 3)
ERYTHROCYTES (X10[6] /MM[3])	7.16 ± .28	7.20 ± .09	6.77 ± .20	7.03 ± .10	7.25 ± .07
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.54 ± .06	.63 ± .10	.59 ± .10	.70 ± .14	.47 ± .06
HEMATOCRIT, VOL. %	47.5 ± 1.3	46.5 ± .6	44.5 ± 1.2	46.8 ± .8	49.0 ± 1.2
HEMOGLOBIN, GM. %	15.8 ± .5	15.6 ± .2	14.9 ± .4	15.9 ± .3	16.2 ± .1
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	66.4 ± 1.1	64.6 ± .4	65.8 ± 1.3	66.5 ± 1.6	67.6 ± 1.8
MCHR, MICRO MICROGMS.	22.1 ± .2	21.7 ± .1	22.1 ± .3	22.7 ± .5	22.4 ± .4
MCHBC, GM %	33.3 ± .4	33.7 ± .3	33.6 ± .7	34.1 ± .3	33.2 ± .7
PLATELETS (X10[5] /MM[3])	6.0 ± .3	6.3 ± .5	6.8 ± .4	5.0 ± .2	7.7 ± .6
LEUKOCYTES (X10[3] /MM[3])	9.9 ± .5	9.8 ± 1.4	10.8 ± .9	11.1 ± .9	14.2 ± 2.1
NEUTROPHILS, %	9.3 ± 3.2	10.0 ± 3.9	14.5 ± 4.1	15.5 ± 1.6	8.0 ± 2.1
LYMPHOCYTES, %	89.8 ± 3.3	88.3 ± 4.1	82.5 ± 4.3	83.1 ± 1.9	90.3 ± 1.2
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .4	1.8 ± 1.0	3.0 ± .9	1.3 ± .5	1.7 ± .9
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 25

LABORATORY DATA OF MALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0.00 (C, 4)	1.00 (T, 4)	3.00 (T, 4)	10.00 (T, 4)	10C[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.10 ± .15	7.24 ± .08	7.17 ± .17	6.63 ± .30	7.25 ± .22 (3)
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.73 ± .19	.40 ± .08	.38 ± .08	.53 ± .09	.45 ± .06
HEMATOCRIT, VOL. %	43.3 ± .6	42.8 ± .3	43.5 ± .6	43.3 ± .5	45.0 ± .6 (3)
HEMOGLOBIN, GM. %	14.0 ± .2	14.4 ± .1	14.4 ± .1	13.9 ± .3	14.9 ± .3 (3)
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
MCV, CUBIC MICRONS	60.9 ± .5	59.1 ± .7	60.7 ± 1.7	65.5 ± 2.3	62.2 ± 1.8 (3)
MCHB, MICRO MICROGMS.	19.8 ± .4	19.9 ± .3	20.1 ± .4	21.0 ± .8	20.6 ± .3 (3)
MCHBC, GM %	32.5 ± .4	33.6 ± .4	33.1 ± .3	32.1 ± .5	33.1 ± .7 (3)
PLATELETS ($X10^5$ /MM^3)	10.8 ± 1.7	7.1 ± .5	6.5 ± .8	6.6 ± .3	7.8 ± 1.6
LEUKOCYTES ($X10^3$ /MM^3)	10.4 ± .7	10.3 ± .6	9.5 ± .3	9.8 ± .8	7.4 ± 1.5 (3)
NEUTROPHILS, %	24.0 ± 2.1	19.5 ± 2.8	25.5 ± 1.8	31.8 ± 6.0	19.3 ± 3.9
LYMPHOCYTES, %	73.0 ± 2.2	76.5 ± 4.7	72.8 ± 1.9	65.5 ± 6.0	80.0 ± 4.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	2.5 ± .3	1.3 ± .5	1.5 ± .5	1.3 ± .3	.8 ± .3[b/]
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.5 ± .3	.3 ± .3	.3 ± .3	1.5 ± .5	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	135.8 ± 5.9	145.3 ± 5.0	133.8 ± 1.3	137.0 ± 5.9	165.3 ± 31.1
SGOT, IU/L	87 ± 12	84 ± 10	57 ± 2	107 ± 13	105 ± 16
SGPT, IU/L	23.5 ± 2.8	23.0 ± 3.8	18.8 ± 1.9	38.3 ± 14.0	32.0 ± 4.8
ALK. PHOS., IU/L	36 ± 3	32 ± 2	30 ± 1	32 ± 3	37 ± 4
CHOLESTEROL, MG %	101 ± 22	111 ± 10	73 ± 9	68 ± 4	75 ± 7
BUN, MG %	15.5 ± 2.5	12.8 ± 1.1	11.0 ± 1.1	12.3 ± .9	14.3 ± .6
IMMUNOGLOBULIN F, IU/ML	2488 ± 820			1150 ± 357	1813 ± 911

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 26

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 12 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0.00 (C, 3)	1.00 (T, 4)	3.00 (T, 4)	10.00 (T, 4)	10C[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.20 ± .07 (2)	6.27 ± .19 (3)	6.52 ± .26	5.81 ± .13	6.19 ± .17 (2)
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.68 ± .27 (2)	.51 ± .21 (3)	.68 ± .49	1.07 ± .26	1.31 ± 1.09 (2)
HEMATOCRIT, VOL. %	41.0 ± 1.0 (2)	39.0 ± 1.7 (3)	39.5 ± 1.2	39.3 ± .5	40.7 ± 2.2 (3)
HEMOGLOBIN, GM. %	13.4 ± .5 (2)	13.4 ± .3 (3)	13.4 ± .3	13.0 ± .2	13.1 ± .3 (2)
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.4 ± .4
MCV, CUBIC MICRONS	66.2 ± 2.4 (2)	62.2 ± 2.2 (3)	60.7 ± 1.7	67.7 ± 2.0	66.9 ± 3.9 (2)
MCHB, MICRO MICROGMS.	21.6 ± 1.1 (2)	21.5 ± .5 (3)	20.6 ± .6	22.4 ± .2	21.2 ± .2 (2)
MCHBC, GM %	32.7 ± .4 (2)	34.5 ± .7 (3)	33.9 ± .3	33.1 ± .9	31.9 ± 2.1 (2)
PLATELETS ($X10^5$ /MM^3)	6.7 ± .5 (2)	5.4 ± .4 (3)	6.5 ± .4	5.3 ± 1.3 (3)	7.1 ± 1.1 (3)
LEUKOCYTES ($X10^3$ /MM^3)	8.3 ± .2 (2)	6.5 ± .5 (3)	5.8 ± .2	8.5 ± 1.2	7.5 ± 2.0 (2)
NEUTROPHILS, %	29.3 ± 2.0	29.0 ± 4.2	29.5 ± 3.1	22.8 ± 3.0	28.3 ± 8.1 (3)
LYMPHOCYTES, %	69.3 ± 2.6	70.3 ± 4.2	69.3 ± 3.2	74.5 ± 3.4	70.3 ± 7.9 (3)
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± .6	.3 ± .3	1.0 ± .4	1.3 ± .3	1.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .3	.3 ± .3	1.5 ± .6	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	133.0 ± 3.8	149.5 ± 9.0	129.0 ± 2.3	127.8 ± 5.0	141.3 ± 12.5
SGOT, IU/L	146 ± 47	93 ± 23	61 ± 11	148 ± 55	209 ± 39
SGPT, IU/L	40.0 ± 14.7	46.8 ± 21.0	28.5 ± 2.8	33.8 ± 5.8	65.8 ± 15.3
ALK. PHOS., IU/L	26 ± 2	17 ± 2	18 ± 1	20 ± 2	25 ± 5
CHOLESTEROL, MG %	116 ± 9	86 ± 7	103 ± 8	89 ± 15	113 ± 5
BUN, MG %	12.0 ± 1.2	12.0 ± .7	11.3 ± 1.3	12.0 ± .8	12.5 ± .6
IMMUNOGLOBULIN E, IU/ML	1500 ± 900 (2)			1838 ± 758	1333 ± 433 (3)

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 27

LABORATORY DATA OF MALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10C[a] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.22 ± .44	7.40 ± .32	7.56 ± .42	7.68 ± .20	7.42 ± .19
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RETICULOCYTES, %	.81 ± .25	.79 ± .17	.82 ± .09	1.31 ± .22	.86 ± .04
HEMATOCRIT, VOL. %	49.8 ± 2.9	48.0 ± 1.9	47.8 ± 1.3	48.8 ± .6	49.3 ± 1.4
HEMOGLOBIN, GM. %	15.9 ± .7	15.2 ± .7	15.7 ± .4	15.2 ± .4	15.9 ± .3
METHEMOGLOBIN, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	.6 ± .4	1.3 ± .0[b]
MCV, CUBIC MICRONS	68.9 ± 1.2	64.9 ± 1.1	63.5 ± 2.3	63.7 ± 2.3	66.4 ± .9
MCHB, MICRO MICROGMS.	22.1 ± .6	20.5 ± .2	20.8 ± .7	19.8 ± .5[b]	21.5 ± .3
MCHBC, GM %	32.1 ± .5	31.6 ± .4	32.8 ± .4	31.1 ± .8	32.4 ± .5
PLATELETS ($X10^5$ /MM^3)	8.8 ± .2	8.8 ± .5	7.8 ± .7	7.7 ± 1.4	7.4 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	14.2 ± 1.5	17.3 ± 3.6	13.5 ± 1.5	15.5 ± 1.8	12.8 ± .6
NEUTROPHILS, %	22.5 ± 6.1	17.3 ± 5.0	19.3 ± 5.4	23.8 ± 6.1	15.8 ± 1.3
LYMPHOCYTES, %	75.3 ± 6.6	79.3 ± 4.7	78.5 ± 5.6	73.3 ± 5.8	80.8 ± 1.3
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	2.5 ± 1.6	1.5 ± .3	1.0 ± .4	1.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.3 ± .6	1.0 ± .7	.8 ± .5	1.8 ± .9	1.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 28

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 18 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.74 ± .26	6.48 ± .49	6.19 ± .15	6.22 ± .19	6.94 ± .15[b/]
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.07 ± .10	.81 ± .19	.92 ± .11	.59 ± .08	1.05 ± .16
HEMATOCRIT, VOL. %	45.8 ± 1.3	45.5 ± .9	47.0 ± 1.3	46.0 ± .6	46.3 ± 1.5 (3)
HEMOGLOBIN, GM. %	14.4 ± .2	14.7 ± .5	15.0 ± .4	14.5 ± .6	14.6 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± .1 (3)[b/]
MCV, CUBIC MICRONS	79.8 ± 1.7	71.3 ± 4.6	76.0 ± 2.5	74.0 ± 1.5	66.9 ± 1.8 (3)[b/]
MCHB, MICRO MICROGMS.	25.1 ± .8	23.1 ± 1.3	24.2 ± .5	23.2 ± .3	21.1 ± .3[b/]
MCHBC, GM %	31.5 ± .6	32.4 ± .6	31.9 ± .6	31.4 ± .9	31.4 ± .4 (3)
PLATELETS ($X10^5$ /MM^3)	7.7 ± .5	7.8 ± .5	8.1 ± .4	7.4 ± .4	7.0 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	11.7 ± 1.9	13.4 ± 2.5	12.9 ± 1.4	9.5 ± 1.2	15.8 ± 2.6
NEUTROPHILS, %	20.0 ± 2.0	22.5 ± 1.5	15.5 ± 4.0	15.3 ± 2.1	21.3 ± 4.0
LYMPHOCYTES, %	78.0 ± 2.7	74.0 ± 1.8	82.8 ± 4.5	80.8 ± 2.1	75.8 ± 3.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.8 ± .9	2.0 ± 0.0	.8 ± .5	2.5 ± .5	1.5 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	1.5 ± .6	1.0 ± .7	1.5 ± .3	1.5 ± .6
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 29

LABORATORY DATA OF MALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N - NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10C[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.88 ± .47	8.18 ± .21	7.02 ± .95	8.00 ± .21	7.70 ± .61
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	2.21 ± .71	1.27 ± .10	1.85 ± .43	1.25 ± .16	2.82 ± 1.90 (3)
HEMATOCRIT, VOL. %	46.3 ± 3.3 (3)	52.0 ± .4	44.5 ± 4.6	51.3 ± 1.3	53.0 ± 1.4
HEMOGLOBIN, GM. %	14.2 ± .6	16.2 ± .2	14.1 ± 1.7	16.2 ± .5	15.9 ± .9
METHEMOGLOBIN, %	.1 ± .1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.6 ± .4
MCV, CUBIC MICRONS	68.0 ± 2.1 (3)	63.6 ± 1.4	64.6 ± 3.2	64.1 ± 1.1	70.3 ± 6.7
MCHB, MICRO MICROGMS.	20.8 ± .8	19.8 ± .4	20.3 ± .6	20.2 ± .2	20.9 ± 1.1
MCHBC, GM %	31.1 ± .5 (3)	31.2 ± .3	31.6 ± .6	31.5 ± .5	30.0 ± 1.5
PLATELETS ($X10^5$ /MM^3)	4.9 ± .7	6.7 ± .6	5.6 ± .4	5.7 ± .3	4.3 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	32.1 ± 12.1	12.5 ± 1.2	11.1 ± .7	14.4 ± 1.9	14.6 ± 1.7
NEUTROPHILS, %	26.8 ± 12.0	31.5 ± 3.2	38.2 ± 7.9	26.0 ± 2.9	20.8 ± 4.5
LYMPHOCYTES, %	55.8 ± 13.8	67.5 ± 2.9	61.8 ± 7.9	73.3 ± 3.2	76.0 ± 4.7
BANDS, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .8	.5 ± .3	0.0 ± 0.0	.3 ± .2	1.2 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	.3 ± .3	2.0 ± .8[b/]
ATYPICAL, %	16.8 ± 16.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	86.5 ± 10.3	85.3 ± 8.0	92.8 ± 13.7	108.0 ± 3.4	78.5 ± 7.2
SGOT, IU/L	72.0 ± 6.2	81.0 ± 4.2	89.0 ± 12.4	64.0 ± 6.0	86.5 ± 4.6
SGPT, IU/L	31.8 ± .8	28.5 ± 1.7	33.0 ± 3.5	26.8 ± 4.4	25.0 ± 2.1
ALK. PHOS., IU/L	44 ± 4	43 ± 5	56 ± 7	40 ± 4	34 ± 5
CHOLESTEROL, MG %	109 ± 16	198 ± 54	109 ± 24	122 ± 18	147 ± 28
BUN, MG %	57.0 ± 43.3	15.3 ± 1.0	13.3 ± .8	15.0 ± .8	13.0 ± .7
IMMUNOGLOBULIN E, IU/ML	1585 ± 590			1198 ± 315	1235 ± 394

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 30

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 4)	10[a/] C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.87 ± .61	6.30 ± 1.35	7.01 ± .17	6.89 ± .47	5.66 ± 1.25
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.08 ± .36	10.34 ± 9.03	.98 ± .34	.86 ± .23	7.30 ± 6.66 (3)
HEMATOCRIT, VOL. %	44.5 ± 2.5	44.0 ± 5.0	47.5 ± 1.0	45.0 ± 2.5	37.0 ± 8.5 (3)
HEMOGLOBIN, GM. %	13.3 ± .6	13.9 ± 2.2	15.0 ± .4	14.8 ± .9	12.4 ± 2.2
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0
MCV, CUBIC MICRONS	77.9 ± 8.0	80.2 ± 16.1	67.8 ± .6	65.6 ± 1.9	79.4 ± 15.3 (3)
MCHB, MICRO MICROGMS.	23.1 ± 1.6	23.9 ± 3.0	21.5 ± .4	21.5 ± .3	23.4 ± 2.3
MCHBC, GM %	30.0 ± 1.1	30.9 ± 1.8	31.7 ± .3	32.9 ± .6	31.2 ± 1.9 (3)
PLATELETS ($X10^5$ /MM^3)	5.6 ± .8	4.7 ± .4	3.9 ± .4	4.1 ± .7 (3)	4.0 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	10.4 ± 1.3	17.4 ± 5.2	13.0 ± 2.8	10.7 ± 2.2	14.9 ± 2.5
NEUTROPHILS, %	24.8 ± 1.4	23.3 ± 4.9	29.8 ± 5.6	37.0 ± 7.9	24.3 ± 5.2
LYMPHOCYTES, %	73.0 ± 2.1	76.3 ± 5.3	67.5 ± 6.2	62.5 ± 7.8	73.8 ± 5.5
BANDS, %	.3 ± .3	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	1.2 ± .8
EOSINOPHILS, %	1.0 ± .6	.3 ± .3	1.2 ± .5	.5 ± .3	.3 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	1.0 ± .6	.5 ± .5	1.2 ± .8	0.0 ± 0.0	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	3.5 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GLUCOSE (FASTING), MG %	117.5 ± 5.7	126.8 ± 10.5	117.5 ± 11.0	125.3 ± 9.9	129.0 ± 8.4
SGOT, IU/L	89.0 ± 11.9	65.2 ± 12.9	63.3 ± 16.4	48.3 ± 2.3	65.5 ± 4.3
SGPT, IU/L	24.3 ± 2.4	26.4 ± 1.7	27.5 ± 4.7	25.3 ± 1.7	29.0 ± 3.1
ALK. PHOS., IU/L	22 ± 2	21 ± 5	30 ± 7	20 ± 6	21 ± 3
CHOLESTEROL, MG %	137 ± 26	110 ± 9	119 ± 11	116 ± 16	125 ± 19
BUN, MG %	20.3 ± 5.0	13.0 ± 1.5	15.0 ± 1.6	13.8 ± .9	14.8 ± .5
IMMUNOGLOBULIN E, IU/ML	2017 ± 240			1763 ± 353	1323 ± 472[b/]

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 31

LABORATORY DATA OF MALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 0)	1 (T, 2)	3 (T, 2)	10 (T, 4)	10C[a/] (C, 2)
ERYTHROCYTES ($X10^6$ /MM^3)		7.37 ± .22	7.44 ± .08	6.71 ± .22	5.81 ± 1.65
HEINZ BODIES, %		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %		.68 ± .16	.36 ± .07	.87 ± .35	8.45 ± 6.88
HEMATOCRIT, VOL. %		45.5 ± .5	46.5 ± 1.5	42.8 ± 1.7	38.0 ± 5.0
HEMOGLOBIN, GM. %		15.0 ± .3	15.1 ± .6	14.4 ± .5	12.3 ± 1.9
METHEMOGLOBIN, %		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS		61.8 ± 1.1	62.5 ± 2.7	63.7 ± .5	68.6 ± 10.9
MCH8, MICRO MICROGMS.		20.4 ± .2	20.3 ± 1.0	21.5 ± .3	22.1 ± 2.9
MCHBC, GM %		33.0 ± .3	32.5 ± .2	33.7 ± .5	32.4 ± .9
PLATELETS ($X10^5$ /MM^3)		4.8 ± .6	5.0 ± 1.0	4.7 ± .6	3.6 ± 1.1
LEUKOCYTES ($X10^3$ /MM^3)		8.0 ± .1	8.3 ± .5	9.0 ± 1.2	7.6 ± .7
NEUTROPHILS, %		37.0 ± 2.0	28.5 ± 4.5	33.8 ± 5.2	55.5 ± 7.5
LYMPHOCYTES, %		61.0 ± 1.0	69.0 ± 5.0	64.3 ± 5.2	44.0 ± 7.0
BANDS, %		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %		1.0 ± 0.0	2.5 ± .5	2.0 ± .4	.5 ± .5
BASOPHILS, %		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %		1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %		0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	22.0 ± 22.0
GLUCOSE (FASTING), MG %		115.0 ± 13.0[b/]	95.5 ± 8.5	104.0 ± 7.0[b/]	65.6 ± 4.6
SGOT, IU/L		62 ± 7[b/]	67 ± 2[b/]	65 ± 5[b/]	101 ± 7
SGPT, IU/L		24.0 ± 0.0	21.0 ± 0.0	22.5 ± 1.5	32.0 ± 8.0
ALK. PHOS., IU/L		53 ± 1	41 ± 10	49 ± 4	27 ± 25
CHOLESTEROL, MG %		117 ± 13	178 ± 38	185 ± 25	124 ± 39
BUN, MG %		13.5 ± .5	16.0 ± 0.0	16.8 ± .9	13.0 (1)
IMMUNOGLOBULIN E, IU/ML				1738 ± 222	1675 ± 225

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 32

LABORATORY DATA OF FEMALE RATS AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS AND ALLOWING TO RECOVER FOR 1 MONTH

(C,N) CONTROL (T,N) TREATED N = NUMBER OF RATS

DOSE: % IN FEED	0 (C, 3)	1 (T, 4)	3 (T, 3)	10 (T, 3)	10[a/] (C, 3)
ERYTHROCYTES ($X10^6$ /MM^3)	5.81 ± .30	6.52 ± .24	6.79 ± .28	6.41 ± .25	5.25 ± .64
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.28 ± .04	1.34 ± .16	1.29 ± .18	1.32 ± .28	2.78 ± 1.67
HEMATOCRIT, VOL. %	41.7 ± 1.3	43.0 ± 1.3	45.0 ± 1.0	43.7 ± 2.9	40.3 ± 3.8
HEMOGLOBIN, GM. %	13.4 ± .2	14.5 ± .4	15.3 ± .4	14.3 ± .8	12.9 ± 1.4
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	72.0 ± 3.6	66.0 ± .8	66.5 ± 2.8	68.0 ± 1.9	77.5 ± 2.9
MCHB, MICRO MICROGMS.	23.2 ± 1.2	22.3 ± .3	22.6 ± 1.0	22.3 ± .5	24.7 ± .6
MCHBC, GM %	32.2 ± .6	33.9 ± .2[b/]	33.9 ± .2[b/]	32.9 ± .3	32.0 ± .5
PLATELETS ($X10^5$ /MM^3)	4.2 ± .5	4.4 ± .4	4.4 ± .3	4.5 ± .3	3.5 ± .4
LEUKOCYTES ($X10^3$ /MM^3)	5.3 ± .4	6.2 ± .7	5.7 ± .4	4.8 ± .1	7.4 ± 1.2
NEUTROPHILS, %	42.3 ± 3.2	32.8 ± 3.3	39.7 ± 1.9	38.7 ± 3.0	31.7 ± 3.2
LYMPHOCYTES, %	56.3 ± 2.4	65.0 ± 2.9	58.3 ± 2.8	61.0 ± 2.6	66.3 ± 3.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	1.0 ± 1.0	1.8 ± .5	2.0 ± 1.0	.3 ± .3	1.7 ± .3
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.3 ± .3	.5 ± .3	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3
GLUCOSE (FASTING), MG %	107.7 ± 11.9	119.8 ± 6.8	111.7 ± 3.2	116.0 ± 9.5	112.0 ± 1.0
SGOT, IU/L	56.3 ± 4.3	54.0 ± 5.4	67.0 ± 12.7	65.7 ± 9.8	67.0 ± 2.6
SGPT, IU/L	25.3 ± 3.0	19.5 ± 1.9	32.7 ± 8.4	18.0 ± 3.0	21.3 ± 4.8
ALK. PHOS., IU/L	31 ± 4	25 ± 3	43 ± 15	21 ± 3	30 ± 7
CHOLESTEROL, MG %	160 ± 33	100 ± 18	134 ± 44	136 ± 5	76 ± 9
BUN, MG %	14.7 ± .9	16.0 ± .7	15.3 ± 1.7	16.3 ± 1.8	14.3 ± .9
IMMUNOGLOBULIN E, IU/ML	1317 ± 120			2103 ± 217[b/]	450 ± 0[b/]

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL RATS (DUNNETT'S MULTIPLE COMPARISON PROCEDURE).

TABLE 33

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED NC FOR 12 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
			Absolute Organ Weight (g)						
Male	0	694 ± 27[b]	2.11 ± 0.06	1.65 ± 0.12	18.1 ± 1.0	4.10 ± 0.18	1.10 ± 0.10	4.03 ± 0.11	
	1	666 ± 21[b]	2.01 ± 0.23	1.83 ± 0.09	14.8 ± 2.5	3.58 ± 0.21	0.87 ± 0.04	3.57 ± 0.04	
	3	669 ± 20[b]	2.05 ± 0.07	1.70 ± 0.10	15.5 ± 1.1	3.85 ± 0.33	0.82 ± 0.02[d]	3.51 ± 0.19[d]	
	10	613 ± 40[b]	2.19 ± 0.04	1.61 ± 0.09	14.5 ± 1.3	3.54 ± 0.21	0.80 ± 0.06[d]	3.72 ± 0.10	
	10C[a]	557 ± 9[b,d]	2.07 ± 0.09	1.46 ± 0.05	12.6 ± 0.6	3.22 ± 0.09[d]	0.73 ± 0.03[d]	3.82 ± 0.15	
Female	0	379 ± 18[c]	2.12 ± 0.05	1.13 ± 0.10	9.3 ± 0.7	2.35 ± 0.11	0.58 ± 0.06		0.147 ± 0.009
	1	400 ± 28[b]	2.08 ± 0.06	1.37 ± 0.19	10.2 ± 0.4	2.19 ± 0.05	0.54 ± 0.05		0.308 ± 0.188
	3	384 ± 16[b]	2.00 ± 0.10	1.28 ± 0.04	9.7 ± 0.4	2.25 ± 0.06	0.62 ± 0.08		0.145 ± 0.016
	10	391 ± 31[b]	2.05 ± 0.07	1.21 ± 0.03	9.4 ± 0.4	2.63 ± 0.23	0.49 ± 0.08		0.145 ± 0.020
	10C[a]	371 ± 25[b]	2.10 ± 0.05	1.07 ± 0.06	9.6 ± 0.6	2.35 ± 0.30	0.61 ± 0.04		0.338 ± 0.204
			Relative Organ Weight (g/100 g body weight)						
Male	0		0.31 ± 0.02	0.24 ± 0.02	2.63 ± 0.23	0.59 ± 0.02	0.16 ± 0.01	0.59 ± 0.04	
	1		0.30 ± 0.04	0.28 ± 0.01	2.22 ± 0.36	0.54 ± 0.04	0.13 ± 0.01	0.54 ± 0.02	
	3		0.31 ± 0.02	0.26 ± 0.02	2.33 ± 0.21	0.58 ± 0.06	0.12 ± 0.00	0.53 ± 0.03	
	10		0.36 ± 0.03	0.27 ± 0.03	2.42 ± 0.14	0.59 ± 0.06	0.13 ± 0.02	0.62 ± 0.05	
	10C		0.37 ± 0.02	0.26 ± 0.01	2.52 ± 0.13	0.58 ± 0.02	0.13 ± 0.01	0.69 ± 0.02	
Female	0		0.58 ± 0.02	0.31 ± 0.03	2.53 ± 0.10	0.64 ± 0.06	0.16 ± 0.01		0.040 ± 0.004
	1		0.53 ± 0.02	0.35 ± 0.06	2.57 ± 0.16	0.55 ± 0.02	0.14 ± 0.01		0.077 ± 0.049
	3		0.52 ± 0.03	0.34 ± 0.02	2.54 ± 0.18	0.59 ± 0.01	0.17 ± 0.03		0.038 ± 0.006
	10		0.54 ± 0.06	0.31 ± 0.02	2.46 ± 0.25	0.68 ± 0.05	0.13 ± 0.02		0.039 ± 0.009
	10C		0.58 ± 0.05	0.29 ± 0.03	2.64 ± 0.31	0.66 ± 0.13	0.17 ± 0.02		0.088 ± 0.052
			Relative Organ Weight (g/g brain weight)						
Male	0		0.78 ± 0.04	8.60 ± 0.64	1.95 ± 0.13	0.53 ± 0.06	1.91 ± 0.04		
	1		0.95 ± 0.10	7.72 ± 1.61	1.84 ± 0.18	0.45 ± 0.06	1.84 ± 0.19		
	3		0.83 ± 0.02	7.50 ± 0.31	1.87 ± 0.11	0.40 ± 0.01	1.71 ± 0.09		
	10		0.73 ± 0.04	6.62 ± 0.61	1.62 ± 0.11	0.37 ± 0.03	1.71 ± 0.06		
	10C		0.71 ± 0.03	6.12 ± 0.43	1.56 ± 0.09	0.36 ± 0.02[d]	1.86 ± 0.12		
Female	0		0.53 ± 0.05	4.40 ± 0.26	1.11 ± 0.07	0.27 ± 0.02			0.070 ± 0.005
	1		0.66 ± 0.11	4.88 ± 0.15	1.05 ± 0.01	0.26 ± 0.02			0.148 ± 0.091
	3		0.65 ± 0.05	4.90 ± 0.43	1.13 ± 0.06	0.31 ± 0.04			0.073 ± 0.010
	10		0.59 ± 0.03	4.58 ± 0.19	1.30 ± 0.15	0.24 ± 0.04			0.071 ± 0.009
	10C		0.51 ± 0.02	4.54 ± 0.23	1.12 ± 0.13	0.29 ± 0.02			0.168 ± 0.106

a/ Fed 10% cotton linters.
b/ Mean ± standard error of four rats.
c/ Mean ± standard error of three rats.
d/ Significantly different from control rats (Dunnett's multiple comparison procedure).

TABLE 34

SUMMARY OF LESIONS OF MALE RATS FED NC FOR 12 MONTHS

Dose (% in feed):	0				10				10C[a/]			
Rat No.:	51-311	51-312	51-313	51-314	54-341	54-342	54-343	54-344	50-301	50-302	50-303	50-304
Lesions[b/]												
Eye												
Corneal dystrophy									4			
Trachea												
Tracheitis	3		1	2	1		3		1			
Lung												
Chronic murine pneumonia	2	2	1	1	2	1	2	1	2	1	2	1
Liver												
Bile duct hyperplasia		1	1	2	1			1				
Portal inflammation or granuloma						1				1		
Hyperplastic foci									1			
Focal necrosis					1			1				
Male Genital System												
Interstitial prostatitis		1										
Kidney												
Nephritis			3	2			1					
Dilated tubules		1			1							
Heart												
Focal myocarditis or fibrosis	1		1	1			1	1			1	
Pancreas												
Focal mononuclear cells infiltration								1				
Bone Marrow												
M/E ratio	2.6	1.5	1.0	2.5	1.3	1.6	2.1	1.1	1.7	1.3	2.0	1.7

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 35

SUMMARY OF LESIONS OF FEMALE RATS FED NC FOR 12 MONTHS

Dose (% in feed):	0			10				10C[a]			
Rat No.:	51-411	51-412	51-414	54-441	54-442	54-443	54-444	50-401	50-402	50-403	50-404
Lesions[b]											
Adrenal Gland											
Cystic degeneration			1	3	1	1	1	1			
Eye											
Retinal atrophy									1		
Trachea											
Tracheitis	3		1			1	3		1		1
Lung											
Chronic murine pneumonia	1	1	1	1	2	2	1	1	1	3	1
Granulomatous pneumonia										1	
Liver											
Portal inflammation or granuloma	1						1		1		1
Telangiectasis								1			
Spleen											
Excessive hemosiderin	1			1	1	2	1	1	1		1
Ovary											
Ovarian cyst					1			1	1		
Uterus											
Endometritis or pyometra				2		1				2	
Endometrial cyst								1			
G. I. Tract											
Intestinal parasitism										1	
Kidney											
Calcinosis						1					
Mammary Gland											
Fibroadenoma									X		
Heart											
Focal myocarditis or fibrosis		1									
Pancreas											
Focal mononuclear cells infiltration					1						
Bone Marrow											
M/E ratio	1.6	1.7	1.4	1.1	1.0	1.2	1.7	2.0	1.5	1.2	1.2

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 36

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF RATS FED NC FOR 24 MONTHS

		Terminal Body Weight (g)	Absolute Organ Weight (g)						
			Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
	0	684 ± 55[b]	2.43 ± 0.08	2.04 ± 0.15	17.3 ± 0.7	4.31 ± 0.85	1.07 ± 0.13	2.49 ± 0.72	
	1	801 ± 45[c]	2.52 ± 0.06	2.15 ± 0.10	18.5 ± 0.9	4.98 ± 0.36	1.19 ± 0.12	3.25 ± 0.21	
	3	703 ± 30[d]	2.44 ± 0.04	2.08 ± 0.05	16.8 ± 0.7	4.69 ± 0.25	1.03 ± 0.04	3.61 ± 0.21	
	10	628 ± 33[e]	2.40 ± 0.07	1.91 ± 0.09	15.6 ± 0.8	4.27 ± 0.29	0.93 ± 0.09	3.21 ± 0.37	
	10C[a]	657 ± 33[f]	2.39 ± 0.02	1.93 ± 0.06	16.2 ± 0.8	4.15 ± 0.19	0.89 ± 0.06	3.23 ± 0.25	
	0	533 ± 40[e]	2.03 ± 0.04	1.49 ± 0.06	14.8 ± 1.3	3.01 ± 0.15	0.75 ± 0.06		0.413 ± 0.185
	1	509 ± 40[g]	2.02 ± 0.04	1.28 ± 0.07	11.9 ± 1.4	2.66 ± 0.30	0.70 ± 0.07		0.229 ± 0.027
	3	464 ± 19[g]	2.07 ± 0.07	1.29 ± 0.05	12.7 ± 1.6	2.52 ± 0.08	0.96 ± 0.11		0.235 ± 0.049
	10	446 ± 20[h]	2.04 ± 0.04	1.34 ± 0.06	11.7 ± 0.9	2.66 ± 0.10	0.78 ± 0.05		0.251 ± 0.064
	10C[a]	515 ± 16[f]	2.03 ± 0.03	1.37 ± 0.05	12.3 ± 0.5	2.73 ± 0.10	0.89 ± 0.15		0.367 ± 0.014
			Relative Organ Weight (g/100 g body weight)						
Male	0		0.36 ± 0.03	0.31 ± 0.04	2.55 ± 0.11	0.63 ± 0.12	0.16 ± 0.01	0.37 ± 0.10	
	1		0.32 ± 0.02	0.27 ± 0.01	2.33 ± 0.12	0.63 ± 0.06	0.15 ± 0.02	0.41 ± 0.02	
	3		0.36 ± 0.02	0.30 ± 0.01	2.42 ± 0.10	0.69 ± 0.06	0.15 ± 0.01	0.52 ± 0.02	
	10		0.39 ± 0.02	0.31 ± 0.01	2.51 ± 0.11	0.70 ± 0.07	0.15 ± 0.01	0.53 ± 0.07	
	10C		0.38 ± 0.02	0.31 ± 0.03	2.53 ± 0.17	0.65 ± 0.05	0.14 ± 0.02	0.51 ± 0.05	
Female	0		0.42 ± 0.04	0.30 ± 0.02	2.95 ± 0.25	0.61 ± 0.05	0.15 ± 0.01		0.072 ± 0.028
	1		0.42 ± 0.03	0.26 ± 0.01	2.34 ± 0.22	0.53 ± 0.04	0.14 ± 0.02		0.046 ± 0.006
	3		0.45 ± 0.02	0.28 ± 0.01	2.70 ± 0.26	0.55 ± 0.02	0.19 ± 0.02		0.051 ± 0.010
	10		0.47 ± 0.02	0.30 ± 0.01	2.62 ± 0.12	0.61 ± 0.03	0.18 ± 0.01		0.058 ± 0.015
	10C		0.40 ± 0.01	0.27 ± 0.01	2.40 ± 0.10	0.54 ± 0.03	0.17 ± 0.02		0.033 ± 0.003
			Relative Organ Weight (g/g body weight)						
Male	0		0.84 ± 0.06	7.12 ± 0.32	1.75 ± 0.30	0.45 ± 0.06	1.00 ± 0.27		
	1		0.85 ± 0.03	7.38 ± 0.43	1.99 ± 0.18	0.47 ± 0.05	1.29 ± 0.07		
	3		0.85 ± 0.02	6.93 ± 0.32	1.93 ± 0.11	0.42 ± 0.02	1.49 ± 0.09		
	10		0.79 ± 0.03	6.51 ± 0.36	1.79 ± 0.13	0.38 ± 0.03	1.33 ± 0.15		
	10C		0.81 ± 0.03	6.79 ± 0.33	1.74 ± 0.07	0.37 ± 0.03	1.35 ± 0.10		
Female	0		0.73 ± 0.03	7.33 ± 0.69	1.49 ± 0.09	0.37 ± 0.05			0.207 ± 0.093
	1		0.63 ± 0.04	5.89 ± 0.72	1.32 ± 0.16	0.35 ± 0.07			0.114 ± 0.015
	3		0.63 ± 0.03	6.15 ± 0.73	1.22 ± 0.03	0.42 ± 0.05			0.116 ± 0.025
	10		0.65 ± 0.03	5.73 ± 0.39	1.30 ± 0.05	0.38 ± 0.02			0.123 ± 0.031
	10C		0.67 ± 0.02	6.06 ± 0.28	1.34 ± 0.05	0.44 ± 0.05			0.082 ± 0.002

a/ Fed 10% cotton linters.
b/ Mean ± standard error of four rats.
c/ Mean ± standard error of five rats.
d/ Mean ± standard error of twelve rats.
e/ Mean ± standard error of eight rats.
f/ Mean ± standard error of eleven rats.
g/ Mean ± standard error of ten rats.
h/ Mean ± standard error of fourteen rats.
i/ Mean ± standard error of nineteen rats.

TABLE 17

SUMMARY OF LESIONS OF CONTROL RATS FED FOR 24 MONTHS

Sex	Male				Female							
	51-	51-	51-	51-	41-	41-	41-	51-	51-	51-	51	51-	51-
BOD N...	194	332	335	322	222	224	225	208	210	211	212	215	221
Lesions[a]													
Adrenal Gland													
Cystic degeneration				1		2	3	3		1	1		3
Cortical adenoma/hyperplasia	X		X						X				
Pituitary													
Adenoma		X				X	X	4				X	X
Thyroid and Parathyroid													
C-cell adenoma								1					
Parafollicular cell hyperplasia	1												
Trachea													
Tracheitis		3	1	1	3				2	1		1	
Lung													
Chronic murine pneumonia	1	1	1	1		1	1	1	1	1	1	1	1
Heart													
Myocardial degeneration and/or fibrosis	2	1	1	1			1			2		1	
Epicarditis											1		
Liver													
Bile duct hyperplasia		1	1	1			1	2	1	1		1	
Portal inflammation		1											
Foci or areas of hepatocellular alteration								1	1			2	
Fatty change (zonal or focal)	3		1						1				
Focal dilated sinusoid			1				1			1			
Spleen													
Hemosiderosis					1	1		1					
Extramedullary hematopoiesis											1		
Testis													
Testicular degeneration and/or atrophy	2	4											
Epididymis													
Epithelial vacuolization	3	1		1									
Prostate													
Suppurative prostatitis				4									
Ovary													
Ovarian cyst									1	1			
Oophoritis										1			
Salpingitis												1	
Uterus													
Endometritis								3		1		1	3
Pancreas													
Focal acinar atrophy	1			2									1
Salivary Gland													
Sialoadenitis	2												
Stomach													
Dilated basal gland		1	1			1							
Calcification of mucosa				2							2		
Ulcer								1					
Intestine													
Chronic enteritis							4						
Mesenteric tumor													X
Kidney													
Hydronephrosis			1	2									
Senile nephropathy or nephritis		1		2						1			1
Focal tubular nephrosis	2	1	1			3	1	1		1		1	2
Calcinosis	2				1	1	1		1	1			
Urinary Bladder													
Mononuclear cell foci in submucosa		1											
Suppurative cystitis				2									
Skin													
Keratoacanthoma	X												
Dermatitis										1			
Brain													
Encephalopathy													1
Eye													
Retinal atrophy/folding						2	1					1	
Keratitis	1												
Mammary Gland													
Fibroadenoma										X	X		
Adenoma						X						X	
Bone Marrow Smear													
M/E ratio	2.3	1.5	1.2	1.4	0	0	0.8	1.3	2.0	1.3	0	0	0

[a] Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ? = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 38

SUMMARY OF LESIONS OF RATS FED 1% NC FOR 24 MONTHS

Sex:	Male					Female							
Rat No.:	42-187	52-136	52-139	52-140	92-146	42-244	42-245	42-248	52-233	52-234	52-239	92-243	52-249
Lesions[a/]													
Adrenal Gland													
Cystic degeneration			1			1		1	2	2	1	3	
Pheochromocytoma								X					
Pituitary													
Adenoma	X		X			X		X		X	X	X	
Thyroid and Parathyroid													
C cell adenoma			X					X	X				
Parafollicular cell hyperplasia											2		
Trachea													
Tracheitis	1			1			1		1	1			
Lung													
Chronic murine pneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1
Bronchiolar adenoma									X				
Heart													
Myocardial degeneration and/or fibrosis	1	1	1	1	1		1	1					
Liver													
Bile duct hyperplasia	1	1	1	1		1	1	1	2			1	
Portal inflammation	1			1			1	1					
Foci or areas of hepatocellular alteration					1	1	2	2				1	3
Fatty change (zonal or focal)			1	1									
Focal dilated sinusoid	1	1									1		
Spleen													
Extramedullary hematopoiesis	1					1			1				
Epididymis													
Epithelial vacuolization	2	3		1	2								
Ovary													
Ovarian cyst								1	1				
Granulosa cell tumor								X					
Uterus													
Endometritis							1	1		1			
Carcinoma									X				
Cystic gland													1
Pancreas													
Focal acinar atrophy					1		1				1		
Stomach													
Dilated basal gland									1				
Intestine													
Chronic enteritis							1						
Kidney													
Senile nephropathy or nephritis	1	2	4					1					
Focal tubular nephrosis	2				2			1					
Calcinosis							1	1		1	1		1
Urinary Bladder													
Lithiasis											1		
Eye													
Retinal atrophy/folding						1				1			
Keratitis										1			
Mammary Gland													
Fibroadenoma						X							
Adenoma						X						X	
Adenocarcinoma						X							
Bone Marrow Smear													
M/E ratio	1.2	1.6	1.4	1.9	1.4	1.7	0	1.0	1.0	0	1.0	0.8	1.0

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ? = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 19

SUMMARY OF LESIONS OF RATS FED 3% NC FOR 24 MONTHS

Sex:	Male												Female								
Rat No.:	43-162	43-163	43-164	43-166	43-168	53-160	53-164	53-168	53-169	53-172	53-173	53-174	43-261	43-263	43-266	53-258	53-259	53-265	53-266	53-269	53-270	53-271
Lesions[a/]																						
Adrenal Glands																						
Cystic degeneration													3	2		3		1	1	2		2
Fatty change (focal)										1												
Pheochromocytoma								X														
Pituitary																						
Adenoma					X					X	X		X			X	X					
Thyroids and Parathyroids																				X	X	X
C cell adenoma		X										X										
Parafollicular cell hyperplasia											X							X				
Trachea																						
Tracheitis	4	3		4	1	3				3	1											
Lungs																						
Chronic murine pneumonia	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1		1	1	1	2	1
Lung abscess																	3					
Heart																						
Myocardial degeneration and/or fibrosis	1	1	1			2		1	2	1	1	1		1	1	1						
Epicarditis																	3					
Liver																						
Bile duct hyperplasia					1	1						1	1			1	1		1	1	2	2
Portal inflammation/granulomas	1		1		1			1							1		1	2	1			
Foci or areas of hepatocellular alteration		1	2	1				1	2			1						1			2	2
Fatty change (zonal or focal)	1		1			2					1						1					
Focal dilated sinusoid		1						1										1				
Cystic bile duct																	1					
Spleen																						
Hemosiderosis														1					1			
Extramedullary hematopoiesis						3			1								2				1	1
Testis																						
Testicular degeneration and/or atrophy								1														
Orchitis										2												
Epididymis																						
Epithelial vacuolization		1	2			1		2		2		2										
Prostate																						
Epithelial vacuolization	1																					
Prostatitis						4				4												
Ovary																						
Ovarian cyst														1			1					
Granulosa cell tumor																			X			
Uterus																						
Endometritis													2			1					2	2
Cystic gland																				1		

(Continued)

TABLE 39 (concluded)

Sex:	Male												Female								
Rat No.:	43-162	43-163	43-164	43-166	43-168	53-160	53-164	53-168	53-169	53-162	53-173	53-174	43-261	43-263	43-266	53-258	53-259	53-265	53-266	53-269	53-270	53-271
Lesions (concluded)																						
Pancreas																						
Focal acinar atrophy							1						1			1		1				
Thymus																						
Malignant lymphoma							X															
Lymph Node																						
Malignant lymphoma							X															
Stomach																						
Dilated basal glands					1												1			1		
Intestine																						
Parasitism		1	1				1			2												
Kidney																						
Hydronephrosis										2												
Senile nephropathy or nephritis	1	2	2				1	2		4		1	1		1			1			1	
Focal tubular nephrosis	1				1			1			1	1				1						
Urinary Bladder																						
Protein plug in lumen			1									1										
Mononuclear cell foci in submucosa										1												
Skeletal Muscle																						
Muscular degeneration	1																					
Eye																						
Keratitis						2		1														
Mammary Gland																						
Fibroadenoma													X				X	X				X
Adenoma													X					X		X		X
Bone Marrow Smear																						
M/E ratio	1.2	1.2	1.1	0.9	1.1	1.1	1.0	1.4	0.8	1.2	1.1	1.5	0.9	1.2	1.1	1.0	1.2	0.8	1.2	1.2	0	1.2

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; A = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 40

SUMMARY OF LESIONS OF RATS FED 10% NC FOR 24 MONTHS

Sex:	Male								Female											
Rat No.:	44-184	44-186	44-187	44-188	54-176	54-190	54-197	54-198	44-283	44-284	44-286	54-283	54-284	54-286	54-287	54-288	54-291	54-292	54-294	54-296	54-297	54-299
Lesions[a/]																						
Adrenal Glands		0						0														
Cystic degeneration	1						3		3	1	3	2	1	3	3	3		2		1		1
Fatty change (focal)				1																		
Cortical adenoma/hyperplasia									X													
Pituitary																						
Adenoma	X	X							X	X	X		X	X		X	X	X	X	X		
Thyroids and Parathyroids																						
C-cell adenoma													X					X				
Parafollicular cell hyperplasia											1											
Trachea																						
Tracheitis			3	3			1		1	2	3	1	1			1		1				
Lungs																						
Chronic murine pneumonia	1	1		1	1	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1
Heart																						
Myocardial degeneration and/or fibrosis	1			1		1	2	1	1										1		1	1
Liver																						
Bile duct hyperplasia		1						1			1								1	1	1	1
Portal inflammation	1	1		1	1	1	1		1	1	1	1		1				1	1		1	1
Foci or areas of hepatocellular alteration						1							1						1			
Fatty change (zonal or focal)		1																				
Focal dilated sinusoid	1												1									
Spleen																						
Hemosiderosis													1	1	1	1	1			1	1	
Extramedullary hematopaiesis									1	2	3		1									
Testis																						
Testicular degeneration and/or atrophy		4																				
Epididymis																						
Epithelial vacuolization			1																			
Ovary																						
Ovarian cyst										1		1		1		1				1	1	1
Oophoritis																	4					
Uterus																						
Endometritis/pyometra									1	1					1		1	1				
Polyps											X	X										
Cystic gland/hydrometra									1			1								1		1

(Continued)

TABLE 40 (concluded)

Sex:	Male								Female											
Rat No.:	44-184	44-186	44-187	44-188	54-176	54-190	54-197	54-198	44-283	44-284	44-286	54-283	54-284	54-286	54-287	54-288	54-291	54-292	54-294	54-296	54-297	54-299
Lesions (concluded)																						
Pancreas																						
Focal acinar atrophy					1	1									1							
Stomach																						
Dilated basal glands													1									
Intestine																						
Parasitism	1					1			1						1							1
Kidney																						
Hydronephrosis					1				3				1									
Senile nephropathy or nephritis				1	1	1	1	1	1		1	1	1	1		1	1		1			
Focal tubular nephrosis		1		1	1	1		1									1	1	1			
Calcinosis									1								1					
Skin																						
Basal cell tumor						X																
Eye																						
Keratitis						1																
Mammary Gland																						
Fibroadenoma												X								X		
Adenoma												X		X								
Adenocarcinoma									X													
Cyst formation										X								X				
Lipoma														X								
Fibroma																					X	
Bone Marrow Smear																						
M/E ratio	0	0	0	0	2.0	1.5	1.2	1.2	1.2	2.0	0.9	1.1	1.2	1.1	1.4	1.0	2.0	0	1.3	1.7	0.9	1.2

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ? = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 41

SUMMARY OF LESIONS OF MALE RATS FED 10% COTTON LINTERS FOR 24 MONTHS

Rat No.:	40-102	40-106	40-107	50-008	50-009	50-016	50-018	50-019	50-022	50-023	50-024
Lesions[a/]											
Adrenal Glands						0					
Cystic degeneration			1								
Fatty change (focal)	1			1	1						
Cortical adenoma/nodular hyperplasia			X	X							
Pituitary											
Adenoma				X			X			X	
Thyroids and Parathyroids											
Parafollicular cell hyperplasia	1			1							
Trachea											
Tracheitis	3		1	4	4	1		1			1
Lungs											
Chronic murine pneumonia	1	1	2	1	1	1	1	1	1	1	1
Heart											
Myocardial degeneration and/or fibrosis	1	1	1	1		1	1	1		1	1
Liver											
Bile duct hyperplasia	1	1						1	1		1
Portal inflammation										1	
Foci or areas of hepatocellular alteration		1			1	1	1	1			
Fatty change (zonal or focal)	1			1							
Hemosiderosis				2							
Spleen											
Hemosiderosis				1							
Extramedullary hematopoiesis			1								
Testis											
Testicular degeneration and/or atrophy			2	4							
Epididymis											
Epithelial vacuolization	1		1				2	1	2		
Foci of mononuclear cells		1									
Pancreas											
Focal acinar atrophy							1				
Islet cell hyperplasia	1										
Salivary Gland											
Sialoademitis	1										
Stomach											
Ulcer				1							
Dilated basal gland									1		
Intestine											
Parasitism			1		1	1				1	
Kidney											
Senile nephropathy or nephritis		1	1				1	1			
Focal tubular nephrosis	2			1	1	1					
Calcinosis						1					
Urinary Bladder											
Mononuclear cells foci in submucosa							1				
Eye											
Retinal atrophy/folding							1	1	1	3	2
Bone Marrow Smear											
M/E ratio	1.8	1.3	1.6	1.8	1.2	2.0	1.7	0	0	0	0

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 42

SUMMARY OF LESIONS OF FEMALE RATS FED 10% COTTON LINTERS FOR 24 MONTHS

Rat No.:	40-202	40-204	40-205	40-207	50-031	50-032	50-033	50-034	50-037	50-038	50-039	50-041	50-042	50-043	50-044	50-045	50-046	50-047	50-048
Lesions[a/]																			
Adrenal Glands																			
Cystic degeneration	1	2		1	1	1	1	3	3	1	1	1	2		1	1	1	1	
Fatty change (focal)				1								1				1			
Cortical adenoma/nodular hyperplasia		X		X					X										
Pheochromocytoma						X				X									
Pituitary																			
Adenoma	X	X		X	X	X	X	X				X		1				X	
Thyroids and Parathyroids																			
C-cell adenoma											X								X
Parafollicular cell hyperplasia	1	1	1			1		1		1								1	
Adenoma of parathyroid								X											
Trachea																			
Tracheitis			1	1			2	1		1	1	1			1			1	
Lungs																			
Chronic murine pneumonia	1	1	1	1	1	2	1	1	2	3	1	1	1	1	1	1	1	1	1
Lung abscess				2						2									
Granulomatous pneumonitis									2										
Heart																			
Myocardial degeneration and/or fibrosis				1			1	1		1		1	1		1	1			
Liver																			
Bile duct hyperplasia		1	1	2					1	1			1	1		1		1	1
Portal inflammation										1			1	2					
Foci or areas of hepatocellular alteration				1						1						2			1
Fatty change (zonal or focal)	1		1		1				1	1	1								
Focal dilated sinusoid							1												
Cystic bile duct																			1
Spleen																			
Hemosiderosis		1	1		1	1	2		1			1				1		1	1
Extramedullary hematopoiesis		1	1											4					
Ovary																			
Ovarian cyst		1		1						1		1							
Oophoritis														4					
Salpingitis										2				4					
Uterus																			
Endometritis	1	1		1		1		2		1		1		1		1		1	
Polyps			X																
Cystic gland	1		1																2

(Continued)

TABLE 42 (concluded)

Rat No.:	40-202	40-204	40-205	40-207	50-031	50-032	50-033	50-034	50-037	50-038	50-039	50-041	50-042	50-043	50-044	50-045	50-046	50-047	50-048
Lesions (concluded)																			
Pancreas																			
Focal acinar atrophy	2		2			1					1								
Islet cell tumor																		X	
Lymph Node																			
Reticuloendothelial cell hyperplasia														4					
Stomach																			
Mucosal calcification										1									
Dilated basal gland											1								
Intestine																			
Parasitism		1						1		1								1	1
Kidney																			
Senile nephropathy or nephritis			1				1		1					2	1			1	
Focal tubular nephrosis	1			1					1					2		1	1		2
Calcinosis			1	1	1			1				1					1	1	1
Urinary Bladder																			
Mononuclear cells foci in submucosa												1							
Skin																			
Dermatitis								1											
Eye																			
Periophthamitis														1					
Retinal atrophy/folding															1	1			
Mammary Gland																			
Fibroadenoma-adenofibroma										X	X				X		X		
Adenoma			X																X
Cyst formation					X														
Bone Marrow Smear																			
M/E ratio	1.8	1.1	1.9	1.3	1.0	1.0	1.4	1.2	0	0	0	0	1.9	0	0.8	1.0	0.9	1.7	1.5

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 43

SUMMARY OF LESIONS OF MALE RATS FED NC FOR 24 MONTHS AND ALLOWED TO RECOVERY FOR 1 MONTH

Dose (% in feed):	1		3		10				10C[a]	
Rat No.:	52-132	52-133	53-157	53-158	54-178	54-180	54-184	54-186	50-001	50-004
Lesions[b]										
Adrenal Glands										
Cystic degeneration			4						1	
Necrosis									3	
Pheochromocytoma								X		
Pituitary										
Adenoma			X							
Thyroid and Parathyroid										
Parafollicular cell hyperplasia	1							1		
Trachea										
Tracheitis		1	4							1
Lung										
Chronic murine pneumonia	1		1	1	1	1	1	1	1	1
Heart										
Myocardial degeneration and/or fibrosis	1	1	1	1	1	2	1	1		
Cardiac dilatation			1	1						
Liver										
Bile duct hyperplasia	1		1	1	1	1	1	1		1
Portal inflammation							1	1		
Eosinophilic or basophilic foci or areas	2	2		1						
Fatty change (zonal or focal)						1			1	
Focal dilated sinusoid		1		1						
Spleen										
Extramedullary hematopoiesis									1	
Testis										
Testicular degeneration and/or atrophy				1		1	4		4	1
Orchitis			1							
Interstitial cell tumor										X
Epididymis										
Epithelial vacuolization		1	1	1					1	
Seminal Vesicle										
Seminal vesiculitis									1	
Pancreas										
Focal acinar atrophy	1	1				1	1	2		
Islet cell hyperplasia	1									
Thymus										
Hemorrhage									1	
Stomach										
Ulceration									2	
Intestine										
Parasitism				1						
Mesenteric tumor									X	
Kidney										
Hydronephrosis			2	2			3			
Senile nephropathy or nephritis	1		3	1	1	1	2	1		1
Focal tubular nephrosis	1	1		1	1	1		1	2	1
Calcinosis					1	1				
Urinary Bladder										
Protein cast									1	
Skin										
Fibroma						X				
Skeletal Muscle										
Muscular degeneration								1		
Brain										
Encephalitis								2		
Ear										
Otitis								3		
Eye										
Keratitis			1				1			
Bone Marrow Smear										
M/E ratio	1.3	1.3	1.0	1.2	1.0	1.0	1.0	1.3	0	0

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 44

SUMMARY OF LESIONS OF FEMALE RATS FED NC FOR 24 MONTHS AND ALLOWED TO RECOVERY FOR 1 MONTH

Dose (% in feed):	0			1				3			10			10C[a]		
Rat No.:	51-202	51-203	51-207	52-226	52-228	52-230	52-232	53-251	53-255	53-257	54-278	54-279	54-280	50-027	50-028	50-029
Lesions[b]																
Adrenal Gland																
Cystic degeneration	1		3	2	1	2	4	1	1	3	3	1	1	2	3	3
Cortical adenoma				X												
Pituitary																
Adenoma				X		X	X			X		X	X			X
Thyroids and Parathyroids																
Adenoma of thyroid							X									X
Parafollicular cell hyperplasia				1												
Trachea																
Tracheitis			1					1			1	1		1		
Lungs																
Chronic murine pneumonia	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
Heart																
Myocardial degeneration and/or fibrosis				1	1	1		1	1	1					1	
Liver																
Bile duct hyperplasia	3	1	1	1	2			2	1	2			1	1	1	1
Portal inflammation						1	1									
Eosinophilic or basophilic foci or areas	1				1			1	1	1					1	
Fatty change (zonal or focal)						1	1									
Focal dilated sinusoid																
Cystic bile duct	1				1						1		1	1		1
Extramedullary hematopoiesis									1							
Spleen																
Extramedullary hematopoiesis						1			2							
Ovary																
Ovarian cyst		1	1		1			1	1		1				1	
Granulosa cell tumor											X					
Oophoritis		4														
Uterus																
Endometritis		1		1	2	1	1				1		1			
Polyps			X					X								
Leiomyoma			X													
Cystic gland		1					1									1
Pancreas																
Focal acinar atrophy		1									1			1		
Islet cell tumor		X														
Intestine																
Parasitism		1														
Kidney																
Senile nephropathy or nephritis		4		2		1							1			
Focal tubular nephrosis	1			2		1			1			1	1			
Calcinosis	1				2	2	1		1			1				1
Mammary Gland																
Fibroadenoma			X							X	X			X		X
Adenoma							X					X				
Adenocarcinoma																X
Cyst formation				X												
Bone Marrow Smear																
M/E ratio	1.5	0.9	1.2	1.3	1.0	1.4	1.0	1.2	1.1	1.6	1..	1.3	1.4	0	1.2	2.3

Tissues not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; + questionable; X = present; 0 = tissue missing or unreadable.

TABLE 45

SUMMARY OF LESIONS OF MALE CONTROL RATS DYING AT UNSCHEDULED TIMES

Rat No.:	41-124	51-109	51-118	51-102	41-126	51-108	41-128	51-121	41-127	51-107	51-114	51-110	51-101
Week of Death:	58	58	61	67	76	78	78	81	81	82	82	97	107[b]
Lesions[a]													
Autolysis		X											
Adrenal Gland													
Cystic degeneration				1		1		1	2				
Cortical adenoma												X	
Malignant lymphoma													X
Pituitary				0	0					0	0		
Adenoma			X				X	X	X			X	X
Trachea													
Tracheitis					4	3					3	4	
Lung													
Chronic murine pneumonia	1		2	3	4	3		1	1	1	1	4	
Reticulum cell sarcoma							X						
Metastatic tumor											X		
Malignant lymphoma													X
Heart													
Focal myocardial degeneration/fibrosis							1	1			1	2	
Malignant lymphoma													X
Liver													
Bile duct hyperplasia	1							1	1				
Portal inflammation				1	1			1		1			
Foci or areas of hepatocellular alteration						1							
Fatty change	1											1	
Necrosis								1					
Reticulum cell sarcoma							X						
Extramedullary hematopoiesis						1							
Malignant lymphoma													X
Spleen													
Hemosiderosis			1							1			
Extramedullary hematopoiesis					1		4	3					
Malignant lymphoma													X
Testis													
Degeneration/atrophy											1	1	
Hypoplasia										1			
Epididymus													
Vacuolization of epithelium								1		1		1	
Prostate													
Atrophy										1		1	1
Seminal Vesicle													
Atrophy										1		1	1

(Continued)

TABLE 45 (concluded)

Rat No.:	41-124	51-109	51-118	51-102	41-126	51-108	41-128	51-121	41-127	51-107	51-114	51-110	51-101
Week of Death:	58	58	61	67	76	78	78	81	81	82	82	97	107[b]
Lesions (concluded)													
Pancreas													
Focal acinar atrophy												1	1
Salivary Gland													
Interstitial edema						1							
Lymph Node													
Reticulum cell sarcoma							X						
Stomach													
Ulcer	1		1									2	
Malignant lymphoma													X
Intestine													
Parasitism				1	1								
Kidney													
Senile nephropathy	1		1	1	4	1		2	1	2	1	4	
Tubular nephrosis							4						2
Reticulum cell sarcoma							X						
Malignant lymphoma													X
Skin													
Reticulum cell sarcoma							X						
Brain													
Metastatic tumor in meninges							X						
Rib													
Metastatic tumor in bone marrow							X						X
Hypoplasia of bone marrow									3				
Eye													
Metastatic tumor							X						
Skeletal muscle													
Fibrosarcoma											X		
Malignant lymphoma													X

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died in week 3 of recovery after 24 months' feeding.

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MAMMALIAN TOXICITY OF MUNITIONS COMPOUNDS. PHASE III. EFFECTS O--ETC(U)
JAN 80 H V ELLIS, J H HAGENSEN, J R HODGSON DAMD17-74-C-4073

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2 OF 3
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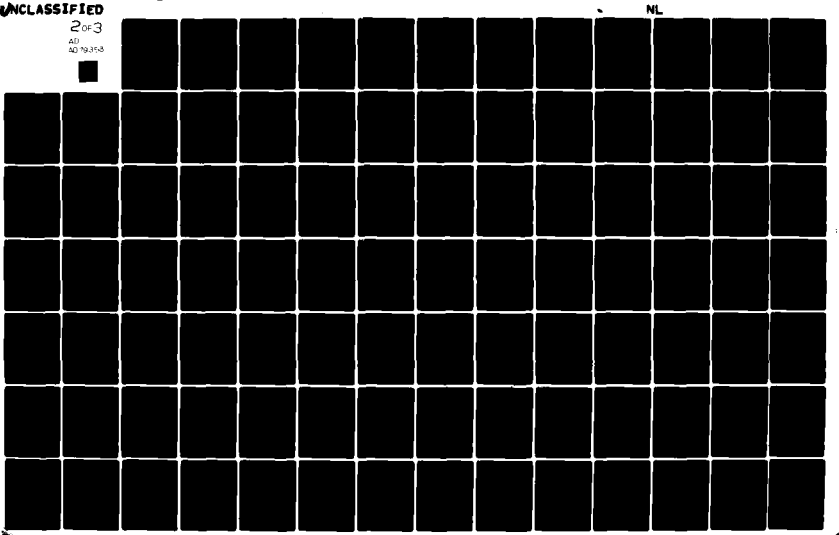

TABLE 46

SUMMARY OF LESIONS OF FEMALE CONTROL RATS DYING AT UNSCHEDULED TIMES

Rat No.:	51-413	51-218	51-206	41-227	51-214	51-219	51-223	41-228	51-213	51-205	41-223	51-224	51-220	51-217	51-204
Week of Death:	50	61	67	75	78	81	82	84	87	87	87	90	102	104	104
Lesions[a]															
Adrenal Gland															
Cystic degeneration				1		3	3	3	4			1	2	1	2
Extramedullary hematopoiesis					2	1									
Pituitary						0				0					
Adenoma		X	X	X				X	X		X	X	X	X	X
Parathyroid															
Adenoma								1							1
Trachea															
Tracheitis	2			1		1	1	3		1		2			
Lung															
Chronic murine pneumonia		1	1			1	1	1	1	1	1	1	1	1	1
Metastatic tumor	X														
Granulomatous pneumonia					2										
Heart															
Ventricle dilatation				3											
Liver															
Bile duct hyperplasia		1		1		1		1			1			2	
Portal inflammation			1							1				1	
Foci or areas of hepatocellular alteration	1														
Fatty change									1					1	1
Necrosis			1		3										
Reticulum cell sarcoma				X											
Extramedullary hematopoiesis					1							1			
Spleen															
Hemosiderosis													1		
Extramedullary hematopoiesis	1			3	3						1	4			1
Ovary															
Ovarian cyst						X									
Uterus															
Endometritis					1		1		2			1	1		1
Cystic hyperplasia of endometrium								2							
Pancreas															
Focal acinar atrophy							1						1		
Reticulum cell sarcoma				1											

(Continued)

TABLE 46 (concluded)

Rat No.:	51-413	51-218	51-206	41-227	51-214	51-219	51-223	41-228	51-213	51-205	41-223	51-224	51-220	51-217	51-204
Week of Death:	50	61	67	75	78	81	82	84	87	87	87	90	102	104	104
Lesions (concluded)															
Lymph Node															
Reticulum cell sarcoma				X											
Stomach															
Ulcer					1	1									
Intestine															
Parasitism									1						
Kidney															
Senile nephropathy			1			1	1		1			1		2	
Tubular nephrosis					2										
Microlithiasis				1	1	1	1	1	1						1
Skin															
Reticulum cell sarcoma				X											
Fibrous histiocytoma						X									
Mammary Gland															
Fibroadenoma									X	X				X	X
Adenoma		X													X
Adenocarcinoma-carcinoma	X	X									X	X			X
Cyst formation							X	X							

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 47

SUMMARY OF LESIONS OF MALE RATS FED 1% NC AND DYING AT UNSCHEDULED TIMES

Rat No.:	52-328	52-149	42-142	52-141	52-137	52-131	52-145	52-144	52-135	52-147	42-141	52-127	52-148	52-143	42-143	52-126
Week of Death:	43	66	67	75	77	78	78	82	87	87	93	99	100	100	103	108[b]
Lesions[a]																
Autolysis	X	X					X			X						
Adrenal Gland															0	
Cystic degeneration			1						1							
Pituitary			0	0				0								
Adenoma											X	X		+	X	
Thyroid and Parathyroid								0								
C cell hyperplasia															X	
Trachea																
Tracheitis	1		1		1	2		2	1		1	1	1			
Lung																
Chronic murine pneumonia	1	3	1	1	1	1		1	1		1	1	1	1		1
Heart																
Myocardial degeneration/fibrosis (focal)				1	1	1			1		1	1	1			1
Ventricle dilatation			1												1	1
Liver																
Bile duct hyperplasia									1			1	1		1	
Portal inflammation			1			1							1		1	
Foci or areas of hepatocellular alteration			1													
Fatty change									1			1				
Necrosis													1			3
Hepatocellular carcinoma		X														X
Proliferation of Kupffer cells														X		
Spleen																
Extramedullary hematopoieses						1		1						X		
Testes																
Periarteritis nodosa																2
Degeneration/atrophy									4						4	
Interstitial edema											1					
Epididymis																
Vacuolization of epithelium			1								2					
Prostate																
Prostatitis												2			1	
Atrophy															1	
Seminal Vesicle																
Seminal vesiculitis												3				
Granuloma														1		
Atrophy															1	

(Continued)

TABLE 47 (concluded)

Rat No.:	52-328	52-149	42-142	52-141	52-137	52-131	52-145	52-144	52-135	52-147	42-141	52-127	52-148	52-143	42-143	52-126
Week of Death:	43	66	67	75	77	78	78	82	87	87	93	99	100	100	103	108[b]
Lesions (concluded)																
Pancreas																
Focal acinar atrophy													2			
Salivary Gland																
Foci of mononuclear cells									1							
Intestine																
Parasitism (pin worm)						1										
Kidney																
Senile nephropathy			1		1				1		1		2	1	1	4
Hydronephrosis													2	3		
Pyelonephritis												2				
Urinary Bladder																
Cystitis												2				
Skin																
Fibroma			X													
Hemangiosarcoma				X		X										
Lipoma						X										
Myxoma									X							
Papilloma													X			
Brain																
Encephalomalacia														2		
Eye																
Keratitis																1
Mammary Gland																
Fibroadenoma					X											

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died in week 4 of recovery after 24 months' feeding.

TABLE 48

SUMMARY OF LESIONS OF FEMALE RATS FED 1% NC AND DYING AT UNSCHEDULED TIMES

Rat No.:	52-426	42-241	52-248	52-249	42-242	52-246	52-244	52-242	52-241	42-246	52-238	52-229	52-236	52-235	52-227	52-247	52-237	42-247	42-243
Week of Death:	51	63	82	82	82	84	87	87	97	97	98	100	101	101	104	102	104	104	104
Lesions[a/]																			
Autolysis	X																		
Adrenal Gland																			
Cystic degeneration									2	2	2	1	3	2	3			1	1
Metastatic tumor		X																	
Pituitary															0				
Adenoma					X	X	X	X	X	X	X	X	X	X		X	X	X	
Thyroid and Parathyroid																			
C cell adenoma																		X	X
Carcinoma							X												
C cell hyperplasia												X							
Trachea																			
Tracheitis			1	1		1		1					1		1				
Lung																			
Chronic murine pneumonia			1	1	1	1	1		1	1	1			1	1	1	1	2	1
Bronchopneumonia	3																		
Metastatic tumor		X										X							
Heart																			
Focal myocardial degeneration/fibrosis					1		1							1		1	1		1
Ventricle dilatation												1							
Blood Vessels																			
Periarteritis nodosa																	3		
Liver																			
Bile duct hyperplasia		1					1	2	1			1		1		1		1	
Portal inflammation																		1	
Foci or areas of hepatocellular alteration				1		1	1		1								1		
Fatty change		3									3	3			1			1	1
Necrosis				1										3					
Hemangiosarcoma														X					
Spleen																			
Hemosiderosis								1	1		1								
Extramedullary hematopoiesis		2									1	4	1	1	1	2	3		1
Ovary																			
Ovarian cyst			X								X	X			X	X			
Abscessation																	4		
Uterus																			
Endometritis					1				1							2	1	1	

(Continued)

TABLE 48 (concluded)

Rat No.:	52-426	42-241	52-248	52-249	42-242	52-246	52-244	52-242	52-241	42-246	52-238	52-229	52-236	52-235	52-227	52-247	52-237	42-247	42-243
Week of Death:	51	63	82	82	82	84	87	87	97	97	98	100	101	101	101	102	104	104	104
Lesions (concluded)																			
Pancreas																			
Focal acinar atrophy													1			3			
Stomach																			
Calcification of mucosa		1																	
Ulcer												1							
Intestine																			
Parasitism																1			
Kidney																			
Senile nephropathy				1		1							1	1	1				
Tubular nephrosis								1		2		2					3		
Microlithiasis					1			1		1		2					1	1	
Calcification		1																	
Skin																			
Lipoma																			X
Brain																			
Encephalomalacia																			1
Eye																			
Retinal atrophy									1										
Mammary Gland																			
Fibroadenoma			X				X									X			
Adenoma			X	X	X								X		X				
Adenocarcinoma-carcinoma		X							X		X		X		X	X			
Cyst formation						X				X									
Mesentery																			
Liposarcoma												X							

TABLE 49

SUMMARY OF LESIONS OF MALE RATS FED 3% NC AND DYING AT UNSCHEDULED TIMES

Rat No.	53-165	53-159	53-152	53-170	53-167	53-151	53-166	53-161	53-163	53-155	53-156
Week of Death:	61	64	66	70	72	80	81	84	85	89	108[b]
Lesions[a]											
Adrenal Gland											
Cystic degeneration			1								
Cortical adenoma	X										
Pituitary		0			0		0	0		0	
Adenoma				X					X		X
Thyroid											
C cell adenoma											X
Trachea											
Tracheitis	1	1					3	1	2		
Lung											
Chronic murine pneumonia	2		1	1		1	1	1		1	1
Pseudotuberculosis					3						
Heart											
Myocardial degeneration/fibrosis		2			1	1	1	1		2	1
Liver											
Bile duct hyperplasia						1			1	1	
Portal inflammation			1	1		2					
Foci or areas of hepatocellular alteration					1					1	
Fatty change	1			1							3
Necrosis		1				1			1		
Extramedullary hematopoiesis							1				
Spleen											
Hemosiderosis				2							
Extramedullary hematopoiesis							3				1
Testis											
Degeneration/atrophy				1							4
Epididymis											
Foci of mononuclear cells							1				
Prostate											
Prostatitis											1
Pancreas											
Focal acinar atrophy											1
Kidney											
Senile nephropathy	1		1	1			1		3		
Tubular nephrosis											2
Microlithiasis	1										
Adenoma				X							
Hydronephrosis								2	1		
Skin											
Myxoma										X	
Trichoepithelioma		X				X					
Preputial gland adenoma				X							
Tumor (unclassified)							X				
Brain											
Encephalomalacia									1		
Eye											
Keratitis			1						1		

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died in week 4 of recovery after 24 months' feeding.

TABLE 50

SUMMARY OF LESIONS OF FEMALE RATS FED 3% NC AND DYING AT UNSCHEDULED TIMES

Rat No.:	43-262	53-256	53-268	53-260	43-268	53-274	53-272	53-253	53-267	43-264	43-267	53-263	53-261	53-252	53-262	53-254	43-265
Week of Death:	53	57	58	59	68	68	71	71	82	82	82	84	87	97	102	102	106[b]
Lesions[a]																	
Autolysis		X															
Adrenal Gland																	
Cystic degeneration			4	1	1	1		2	4	1	3		3		2	3	
Cortical adenoma														X			
Cortical hyperplasia (nodular)					X									X			
Pituitary											0						
Adenoma									X			X		X	X		X
Parathyroid																	
Hyperplasia										X							
Trachea																	
Tracheitis					1	1		1			3	1	3		1		
Lung																	
Chronic murine pneumonia	1		1	1		1	1			1		1	1	1	2	1	1
Granulomatous pneumonia						1											
Heart																	
Myocardial degeneration/fibrosis			1														1
Liver																	
Bile duct hyperplasia											2	1				1	
Portal inflammation					1			1	1			1					1
Foci or areas of hepatocellular alteration											2						
Fatty change													1			1	2
Necrosis														1			
Spleen																	
Hemosiderosis						1		2				1			1		
Extramedullary hematopoiesis	3													2			
Ovary																	
Ovarian cyst																	X
Granulosa cell tumor										X							
Uterus																	
Endometritis			1		1		1		1			1		2			
Polyps																X	
Pancreas																	
Focal acinar atrophy											1			1			
Intestine																	
Parasitism (pin worm)	1											1			1		
Kidney																	
Senile nephropathy			1		1			1	1								
Microlithiasis			1		1	1			1	1	1	1		1		1	1
Urinary Bladder																	
Foci or monomuclear cells in submucosa				1													
Mammary Gland																	
Fibroadenoma			X		X						X		X	X			
Adenoma	X			X	X		X							X		X	
Adenocarcinoma-carcinoma					X	X											
Fibroma										X							
Joint																	
Arthritis								X									

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; + = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died in week 2 of recovery after 24 months' feeding.

TABLE 51

SUMMARY OF LESIONS OF MALE RATS FED 10% NC AND DYING AT UNSCHEDULED TIMES

Rat No.:	54-181	54-193	54-183	44-181	54-179	54-177	54-182	54-192	54-185	44-182	54-187
Week of Death:	68	71	74	76	79	88	90	97	97	97	103
Lesions[a/]											
Adrenal Gland											
Cystic degeneration									1		
Cortical hyperplasia (nodular)										X	
Pituitary					C					C	
Adenoma	X					X	X	X	2		
Parathyroid											
Hyperplasia									X		
Trachea											
Tracheitis	3					1		1			1
Lung											
Acute bronchopneumonia				3							
Chronic murine pneumonia		1			1	1	1	1		1	2
Pseudotuberculosis			4								
Lung abscess							3				
Granuloma		1									
Uremic calcification									2		
Heart											
Ventricle dilatation					3						
Myocardial degeneration/fibrosis		1	2		1	1	1	1	3	1	
Blood Vessels											
Calcification									3		
Liver											
Bile duct hyperplasia		1				1	1		1	1	
Portal inflammation								1		1	1
Fatty change											2
Necrosis				3							
Spleen											
Hemosiderosis									1		
Extramedullary hematopoiesis										1	1
Testes											
Degeneration/necrosis	3	1									
Epididymis											
Foci of mononuclear cells		1									
Vacuolization of epithelium						1	1			1	
Sperm granuloma										X	
Prostate											
Prostatitis (suppurative)									4		
Atrophy											1
Seminal Vesicle											
Seminal vesiculatis (suppurative)									4		
Carcinoma										X	
Pancreas											
Hyperplasia of islet cells								X			
Stomach											
Hyperkeratosis											1
Intestine											
Parasitism (pinworm)				1							
Kidney											
Senile nephropathy					1		2	1			
Focal tubular nephrosis											1
Hydronephrosis	1										
Microlithiasis	1										
Calcification									2		
Urinary Bladder											
Necrotizing cystitis									3		
Skin											
Fibroma/sarcoma		X									
Histiocytoma										X	
Brain											
Meningioma											X
Eye											
Keratitis									1		
Mediastineum											
Caratid body tumor							X				

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ± = questionable; X = present; C = tissue missing or unreadable.

TABLE 52

SUMMARY OF LESIONS OF FEMALE RATS FED 10% NC AND DYING AT UNSCHEDULED TIMES

Rat No.:	54-293	44-287	54-277	44-282	54-298	54-289	54-285	54-281	44-288	54-295	44-281	54-290	54-276	54-282
Week of Death:	63	67	77	78	82	82	83	84	84	87	87	90	104	108[b]
Lesions[a]														
Adrenal Gland														
Cystic degeneration		2		2			4		1	1	3		2	2
Cortical hyperplasia (nodular)		X										X		
Pituitary			0			0						0		
Adenoma					X				X	X	X		X	X
Trachea														
Tracheitis		1		1									3	3
Lung														
Chronic murine pneumonia	1	3	1	1	1	1	1	1	1	2	1	1	1	1
Lung abscess				1										
Heart														
Ventricle dilatation						3	1							
Myocardial degeneration/fibrosis											1			
Liver														
Bile duct hyperplasia										2	1	1	1	
Portal inflammation			1											
Foci or areas of hepatocellular alteration										1		1		
Fatty change	3					3						1		
Fibrosarcoma							X							
Spleen														
Hemosiderosis	1			1	1	1		1						2
Extramedullary hematopoiesis		4	3									1		
Uterus														
Endometritis			1							2				
Polyps											X		X	
Pancreas														
Focal acinar atrophy			1										1	
Stomach														
Ulcer						1								
Intestine														
Parasitism (pin worm)				3										
Kidney														
Senile nephropathy					1	1							1	
Focal tubular nephrosis														2
Microlithiasis											1	1	1	1
Urinary Bladder														
Foci of mononuclear cells in submucosa				1	1									
Skin														
Fibroma/sarcoma							X							
Abscess	X													
Brain														
Encephalomalacia	1													
Eye														
Panophthalmitis						4								
Mammary Gland														
Fibroadenoma			X	X				X				X		
Adenoma														X
Adenocarcinoma-carcinoma		X			X						X			

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ? = questionable; X = present; - = tissue missing or unreadable.

b/ Died in week 4 of recovery after 24 months' feeding.

TABLE 53

SUMMARY OF LESIONS OF RATS FED 10% LINTERS AND DYING AT UNSCHEDULED TIMES

Sex:	Male										Female									
	50-	40-	50-	50-	50-	50-	50-	40-	50-	50-	50-	50-	40-	40-	40-	50-	40-	50-	40-	40-	50-	50-	40-	50-
Rat No.:	002	108	014	007	012	020	011	103	003	013	010	015	101	104	105	049	203	040	201	208	036	030	206	026
Week of Death:	78	78	82	83	87	92	93	93	95	96	100	101	104	104	104	61	68	82	84	87	94	101	104	107[b]
Lesions[a]																								
Autolysis		X																						
Adrenal Gland																								
Cystic degeneration				1			1	1										1	1	1		1	1	1
Focal necrosis	1														3									
Cortical adenoma													X											
Pituitary								0																
Chromophobe adenoma			X			X				X		X						X		X	X	X	X	X
Thyroid and Parathyroid																								
C cell adenoma														X										
Trachea																								
Tracheitis	3		2	4	1	1	1	2	2	3			4	1	4	1		3	3		1		3	1
Lung																								
Chronic murine pneumonia			1	3	3	1	1	2	1	1		1	1	1		2	1		2	1	2	1	2	
Pseudotuberculosis	4														4									
Heart																								
Myocardial degeneration/fibrosis					1			1	1	1				1										
Ventricle dilatation												1										1		
Epicarditis	2														2									
Liver																								
Bile duct hyperplasia	3			1	1		1							1	2	1						1	1	1
Portal inflammation				1			1			1		1	1		1							1		1
Foci or areas of hepatocellular alteration							1																	
Fatty change			3					1		1				1	1			3						
Hepatocellular carcinoma	X																							
Focal necrosis						1																		
Spleen																								
Hemosiderosis				1		1						1	1							1		1	1	
Extramedullary hematopoiesis							1	1		1				1			3				1	1		
Lymphoid depletion																		3						1
Epididymis																								
Vacuolization of epithelium									1					1	2									
Prostate																								
Prostatitis (suppurative)												4												

(Continued)

TABLE 53 (concluded)

Sex:	Male										Female									
	50-	40-	50-	50-	50-	50-	50-	40-	50-	50-	50-	50-	40-	40-	40-	50-	40-	50-	40-	40-	50-	50-	40-	50-
Rat No.:	002	108	014	007	012	020	011	103	003	013	010	015	101	104	105	049	203	040	201	208	036	030	206	026
Week of Death:	78	78	82	83	87	92	93	93	95	96	100	101	104	104	104	61	68	82	84	87	94	101	104	107[b]
Lesions (concluded)																								
Ovary																								
Ovarian cyst																								X
Uterus																								
Endometritis																		3						3
Cystic gland																							1	
Pancreas																								
Hyperplasia of islet cells							1																	
Focal acinar atrophy								1				1											1	
Stomach																								
Ulcer															1									2
Calcification in mucosa	2								1															2
Intestine																								
Parasitism (pinworm)					1		1						1	1										
Kidney																								
Senile nephropathy				1		1	1																	
Calcification	2																							
Focal tubular nephrosis			1																					
Pyelitis												2												
Pseudotuberculosis															2									
Urinary Bladder																								
Cystitis												1												
Skin																								
Fibroma/fibrosarcoma				X										X										
Epidermal cyst									X															
Squamous cell carcinoma																		X						
Brain																								
Abscess									X															
Encephalomalacia											X													
Rib																								
Hypocellularity of bone marrow			3																			1		
Eye																								
Retinal atrophy														1					1					
Mammary Gland																								
Fibroadenoma																					X		X	
Adenoma																	X				X			X
Adenocarcinoma-carcinoma																X				X				
Teeth																								
Malocclusion								X																

Tissues not listed were normal.

a/ Severity of lesions: 1 = mild; 2 = moderate; 3 = severe; 4 = very severe; ? = questionable; X = present; 0 = tissue missing or unreadable.

b/ Died in week 3 of recovery after 24 months' feeding.

TABLE 54

TUMORS IN RATS FED NC

Dose (% in feed):	0		1		5		10		10[a]	
Sex:	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Tumors[b]										
Adrenal Gland										
Cortical adenoma	3[b]	1		1	1	1		1	3	3
Pheochromocytoma	1			1	1		1			2
Pituitary Gland										
Chromophobe cell adenoma	6	10	3	16	6	12	6	18	7	16
Thyroid Gland										
C cell adenoma		1	1	5	3			2	1	3
Carcinoma				1						
Parathyroid Gland										
Adenoma										1
Lung										
Bronchiolar adenoma	1			1						
Liver										
Hemangiosarcoma				1						
Hepatocellular carcinoma			2						1	
Pancreas										
Islet cell tumor		1								1
Testis										
Interstitial cell tumor									1	
Seminal Vesicle										
Carcinoma							1			
Ovary										
Granulosa cell tumor				1		2				
Uterus										
Leiomyoma		1		1						
Kidney										
Adenoma					1					
Skin and Other Connective Tissue										
Histiocytoma		1					1			
Carotid body tumor							1			
Myxoma			1		1					
Keratoacanthoma	1									
Papilloma					1					
Trichoepithelioma					2					
Preputial gland adenoma					1					
Basal cell tumor							1			
Lipoma			1	1						
Liposarcoma				1						
Hemangiosarcoma					2					
Fibroma/fibrosarcoma			1				2	1	2	
Squamous cell carcinoma										1
Multiple Sites										
Malignant lymphoma	1				1					
Reticulum cell sarcoma	1	2								
Brain										
Meningioma							1			
Skeletal Muscle										
Fibrosarcoma	1									
Mammary Gland										
Adenoma		4		8		6		8		5
Fibroadenoma		7	1	4		10		7		5
Fibroma						1		1		
Lipoma								1		
Adenocarcinoma/carcinoma		6		6		2		4		7

a/ Fed 10% cotton linters.
b/ Number of rats.

TABLE 55

AGE, WEIGHT AND FERTILITY OF THREE GENERATIONS OF RATS GIVEN NITROCELLULOSE

Nitrocellulose (% in feed)	Generation	Age at First Mating (months)	Mating Ratio[a]	Pregnancy Ratio[b]	Males: Fertile Mated	Males: Weight (g) at First Mating	Females: Fertile Mated	Females: Weight (g) at First Mating	Duration of Gestation (days)
0	F0	8	25/36	15/25	9/10	695 ± 17[c]	11/20	307 ± 7 (17)	22.0
	F1	3	33/38	32/33	16/16	415 ± 12	18/20	271 ± 7 (15)	21.9
	F2	3	36/40	31/36	20/20	474 ± 7	18/20	273 ± 6 (20)	21.8
1	F0	8	26/37	16/26	7/10	655 ± 21	10/20	338 ± 7 (18)[d]	22.0
	F1	3	40/40[e]	35/40	19/20	456 ± 13[d]	19/20	263 ± 6 (20)	21.7
	F2	3	35/39	31/35	20/20	504 ± 12	20/20	289 ± 5 (20)	21.8
3	F0	8	34/39[e]	17/34	8/10	657 ± 23	11/20	323 ± 6 (21)	22.3
	F1	3	40/40[e]	31/40[e]	18/20	438 ± 7	18/20	319 ± 6 (20)[d]	22.1
	F2	3	39/40	34/39	19/20	476 ± 14	18/20	274 ± 6 (20)	21.8
10	F0	8	29/34	27/29[e]	9/9	547 ± 19[d,f]	17/18[e]	297 ± 6 (19)	21.9
	F1	3	36/36[e]	35/36	18/18	291 ± 5[d,f]	18/18	285 ± 5 (18)[d]	22.1
	F2	3	39/40	35/39	20/20	390 ± 9[d,f]	20/20	265 ± 7 (20)	21.7
10C[g]	F0	8	33/39[e]	29/33[e]	10/10	473 ± 9[d]	17/20[e]	279 ± 4 (16)[d]	21.9
	F1	3	38/40	38/38	20/20	253 ± 5[d]	20/20	231 ± 4 (19)[d]	21.9
	F2	3	38/40	36/38	19/20	247 ± 5[d]	20/20	247 ± 6 (17)[d]	21.7

a/ Number of copulations detected by vaginal smear to the number of male-female pairings.
b/ Number of confirmed pregnancies to the number of copulations.
c/ Mean ± S.E.
d/ Significantly different from the mean value of the respective control generation (Dunnett's multiple comparison procedure).
e/ Significantly different from the ratio for the respective control generation (Fisher exact probability test with Tocher's modification).
f/ Significantly different from the mean value of the respective cotton linter control group (Dunnett's multiple comparison procedure).
g/ Fed 10% cotton linters.

TABLE 56

REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN NITROCELLULOSE IN A THREE-GENERATION STUDY

Nitrocellulose (in Feed)	Litter Number	Litter Size	Liveborn Index	Weight at Birth	Viability Index	Lactation Index	Weight at Weaning	Sex Ratio Males:Total
0	F1a	9.3 ± 1.1(7)[a]	95 ± 4	6.3 ± 0.3	98 ± 2	86 ± 14	47 ± 4(6)	27:49
	F1b	13.2 ± 1.1(4)	96 ± 2	6.3 ± 0.3	96 ± 8	100	44 ± 1	21:48
	F2a	14.4 ± 0.6(14)	100	6.7 ± 0.2	99 ± 1	98 ± 1	42 ± 1	96:194
	F2b	11.0 ± 1.0(14)	98 ± 1	7.3 ± 0.4	99 ± 1	100	50 ± 2	73:148
	F3a	[illegible] ± 0.7(15)	99 ± 1	6.7 ± 0.1	98 ± 1	96 ± 2	42 ± 2	97:196
	F3b	14.6 ± 0.7(14)	98 ± 1	6.6 ± 0.1	98 ± 1	97 ± 1	38 ± 2	92:190
1	F1a	7.8 ± 2.0(8)	86 ± 12	7.5 ± 0.7	82 ± 14(7)	100(6)	53 ± 5(6)	30:57
	F1b	14.2 ± 1.2(6)	100	7.2 ± 0.6	91 ± 9	99 ± 1	49 ± 4	34:76
	F2a	11.6 ± 0.9(16)	100	6.9 ± 0.3	101 ± 1	94 ± 3	52 ± 3	69:175
	F2b	10.0 ± 1.0(16)	93 ± 4	7.0 ± 0.2	99 ± 1	98 ± 2	53 ± 2	77:149
	F3a	13.1 ± 0.5(17)	99 ± 1	6.6 ± 0.1	98 ± 2	97 ± 3	46 ± 1	102:209
	F3b	12.3 ± 1.2(13)	83 ± 10	5.6 ± 0.7	92 ± 8(12)	93 ± 5(11)	39 ± 2	72:140
3	F1a	7.4 ± 1.2(7)	93 ± 7	7.5 ± 0.4	100	95 ± 2	52 ± 3	24:45
	F1b	10.2 ± 1.6(6)	94 ± 6	7.2 ± 0.2	96 ± 4	96 ± 2	56 ± 5	21:52
	F2a	12.4 ± 0.9(16)	100	7.1 ± 0.4	95 ± 2	89 ± 4	50 ± 2	85:169
	F2b	11.4 ± 1.0(13)	94 ± 3	6.6 ± 0.2	96 ± 2	90 ± 4	48 ± 3	62:121
	F3a	13.1 ± 0.5(17)	98 ± 1	6.5 ± 0.1	100	98 ± 2	45 ± 2	97:212
	F3b	12.9 ± 0.8(16)	99 ± 1	6.4 ± 0.1	99 ± 1	91 ± 3	44 ± 2	106:183
10	F1a	9.5 ± 1.7(17)	98 ± 2	7.2 ± 0.6	97 ± 2	82 ± 8	41 ± 8(15)	55:116
	F1b	10.2 ± 1.2(10)	100	7.9 ± 0.4[b]	99 ± 1	73 ± 10	26 ± 3(9)[b]	39:76
	F2a	12.5 ± 0.5(18)	100	6.9 ± 0.1	98 ± 1	51 ± 9[b]	23 ± 3[b]	44:103
	F2b	10.1 ± 1.2(17)	100	7.2 ± 0.4	100 ± 10	56 ± 9[b]	34 ± 4[b]	50:102
	F3a	12.4 ± 0.6(18)	99 ± 1	6.7 ± 0.2	94 ± 6	98 ± 1(17)	42 ± 2	100:205
	F3b	13.8 ± 0.6(15)	97 ± 1	6.2 ± 0.4	91 ± 7	98 ± 1(14)	35 ± 2	81:176
10C[c]	F1a	6.6 ± 1.1(13)	92 ± 5	6.3 ± 0.3	88 ± 8	66 ± 12(12)	27 ± 3(9)	22:48
	F1b	10.7 ± 0.6(12)	95 ± 4	6.8 ± 0.2	98 ± 1	70 ± 11	24 ± 3(10)[b]	42:79
	F2a	14.1 ± 0.6(19)	100	6.7 ± 0.2	98 ± 1	66 ± 7[b]	24 ± 2[b]	78:166
	F2b	12.7 ± 0.9(17)	99 ± 1	6.5 ± 0.2	100	80 ± 8	25 ± 3[b]	69:158
	F3a	11.9 ± 0.7(16)	93 ± 5	6.6 ± 0.1	99 ± 1	99 ± 1	43 ± 3	90:179
	F3b	13.8 ± 0.7(16)	99 ± 1	6.6 ± 0.1	99 ± 1	90 ± 2	37 ± 3	104:193

a/ Mean ± S.E. and in parentheses the number of litters included in the mean.
b/ Signficantly different from the mean value of the respective control litter (Tukey's omega procedure).
c/ Fed 10% cotton linters.

TABLE 57

CHROMOSOMES DERIVED FROM RATS FED NC FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromosome Frequency					Tetraploids per 100 Cells
			≤ 40	41	42	43	≥ 44	
10C[a/]	Bone marrow	4	1[b/]	5	42	1	1	0.12 ± 0.12[c/]
10C	Kidney	4	4	6	37	2	1	0.50 ± 0.20
10	Bone marrow	6	2	2	43	2	1	0.00 ± 0.00
10	Kidney	5	4	7	35	3	1	0.30 ± 0.20

a/ Fed 10% cotton linters.
b/ Mean.
c/ Mean ± standard error.

TABLE 58

MORPHOLOGICAL ABERRATIONS OF CHROMOSOMES DERIVED FROM RATS FED NC FOR 24 MONTHS

Dose (% in feed)	Tissue Cultured	Number of Rats	Chromatid Breaks and Gaps per 50 Cells	Translocations per 50 Cells	Total Aberrations per 50 Cells
10C[a/]	Bone marrow	4	0.5 ± 0.3[b/]	0	0.5 ± 0.3
10C	Kidney	4	3.5 ± 1.0	0	3.5 ± 1.0
10	Bone marrow	6	0.7 ± 0.2	0	0.7 ± 0.2
10	Kidney	5	0.8 ± 0.6	0	0.8 ± 0.6

a/ Fed 10% cotton linters.
b/ Mean ± standard error.

V. MOUSE STUDIES

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V. MOUSE STUDIES

The following sections describe the results and interpretations of the mouse studies.

A. Observations and Toxic Signs

The first observations were of problems with the feeders; these are discussed in the section "Feed Consumption." In the first 3 weeks of the study, nine high-dose (10% NC) males and five high-dose females, five cotton control (10% cotton linters) males, and one cotton control female were found dead. Gross necropsy found emboli of white fibrous material blocking the intestines at various sites from the jejunum downward. This condition was due to these individuals' inability to handle the fiber content of the feed, and was reported earlier.[6] Additional mice, fed the respective diets from the start of the study, were substituted for these dead mice. There were occasional fights and injuries, especially among the males, in the first few weeks. These were controlled by putting the aggressor in a separate cage.

In week 18, we first saw an unusual hyperemia in a cotton control male. His ears and eyelids were hyperemic. His genitalia and later his feet and tail were edemic. In subsequent weeks, this same effect was seen in other mice. It was most common in cotton controls, less common in high dose mice, and almost unknown in the others. It did not correlate with weight loss or unscheduled deaths. Incidence decreased by the end of Month 10. The cause may be an infestation with a mite or fungus of some kind. However, the condition was seen in some mice in cages in the top and bottom rows, with very few cases in other mice in the same cages and in the other rows. Therefore, this is unlikely to be the cause. An alternate explanation is sunburn. However, our lighting is Westinghouse "cool white" fluorescent lights, which emit very little ultraviolet. In addition, the polycarbonate cages and cellulose filter tops are almost opaque to ultraviolet. Finally, the affected animals were the cotton control (top row of cages) and high dose (bottom row) groups, not the other groups (intermediate rows). This hypothesis can also be discarded. The one common factor in the affected mice was the presence of fiber--cotton linters or NC. As discussed below, the cotton control and high dose groups were notorious for pulling the fiber from the feeders, playing with it, and even nesting in it. A hypersensitivity reaction is unlikely, since there was no supporting evidence, such as increased serum IgE, in any species. But an irritation reaction is quite possible. A few of the mice developed sores which did not heal readily, due to continued scratching, an action which implies irritation.

During the study, there were a number of unscheduled deaths, as summarized in Figures 9 and 10. If possible, we took blood samples, and killed the mouse for necropsy. Many of these deaths occurred at night. These tissues were often lost to autolysis, which occurs much more quickly in mice than in larger animals. Cannibalization by cage mates also destroyed tissues before histopathological examination. Among the control, low (1% NC) and middle (3% NC) dose mice, there were few deaths in the first year, and an increasing rate throughout the second year of the study. About Month 9, we had many deaths in the high dose mice and a smaller number in the cotton controls. There was no apparent cause. The deaths occurred one or two at a time, in various cages, without obvious premonitory signs. There were transient decreases of a few grams body weight in some mice which died, and in some which survived. A few mice had obvious tumors, but these were relatively rare and distributed among all groups.

B. Body Weight

Body weights of male and female mice fed NC are shown in Figures 11 and 12, respectively. Some data are omitted for clarity. Normal control mice gained weight quickly, then leveled out near 44 g for males or 37 g for females after 6 months feeding. Low and middle dose mice were much like the normal control mice.

Mice fed the high dose or an equal amount of cotton linters lost weight in the first week, but then began to gain. The gains leveled off near 42 g for males or 30 g for females after 4 months feeding. During the second year, the average weights of all dosage groups converged.

C. Feed Consumption and NC Intake

Measurements of the apparent feed consumption are shown in Figures 13 and 14; overall averages are in Table 59. During the first week of the study, feed consumptions were lower than the expected 5 g/mouse/day. Because of the water in the feed, the feed would harden into a relatively solid mass which the mice could not extract through the grid tops of the feeder. Therefore, we omitted the grids from the second week on to prevent starvation. The mice immediately started playing with the feed, scattering it about the cage. Since we measured the change in feeder weight, this gave us very high apparent consumptions in all dosage groups. Since these figures had little biological significance, measurements were omitted for Months 4 through 7.

However, as the study continued, the behavior of the mice changed. The normal control, low dose and middle dose mice ate normal amounts of the diet. In fact, there was a reasonable dose relationship between the consumptions (see Table 59) as one would expect if the NC were merely inert bulk. The cotton control and high dose cages could still be identified by the fiber scattered within the cages. Thus, the figures remain "apparent" consumption.

We twice attempted to quantitate the actual amount of scattering. After several false starts, we would hold the cages, bedding and spilled feed and fiber after a week of feed measurement. By tedious sifting and plucking, we removed the fiber and weighed it; the feed could be sifted free but was contaminated by fine remnants of the bedding. From the apparent feed consumption, we calculated the fiber available to the mice, and compared this amount with the amount of fiber retrieved. In the high dose cages, about 10% of the fiber was wasted, so actual consumption was probably 90% of apparent consumption. However, we retrieved much more of the linters from the bedding of the cotton control cages. This percentage (0 to over 100%) was extremely variable. The linters arrived baled; they cannot be broken up to individual fibers as readily as the NC fibers. Thus, there was irregularity of mixing (allowing the over 100% recoveries). Furthermore, the larger "pills" of linters were easier for the mice to sort out and to eat around. Therefore, the estimates of linter consumption are not reliable.

D. Laboratory Data

Laboratory data from mice of unscheduled terminations are shown in Table 60 and from mice killed after 12 or 24 months feeding in Tables 61 through 64. There were no consistent, NC-related effects. There were some statistically significant differences, but the differences were small and did not persist through the study. The differences represented normal variation among the mice, rather than toxicologically important differences. The minimal amounts of methemoglobin seen in a few mice of all dosage groups are artifacts of the method, which measures a difference in absorption.

Results from mice allowed to recover for a month after feeding for 24 months are given in Tables 65 and 66. No male cotton control mice survived. The data showed the same natural variations as described above, but none were statistically different.

E. Pathology

Data are presented on 20 male and 21 female control mice and 24 male and 18 female high dose mice. Omissions include recovery mice and those lost to decomposition, cannibalism, etc.

1. Feeding for 12 Months

Average organ weights of mice fed NC for 12 months are given in Table 67. The only statistical differences among the treatment groups were large spleens and ovaries in the cotton control females. Since similar increases were not seen in the males or at other necropsies, these increases are presumably normal variations, unrelated to the treatment.

Lesions in male and female mice fed NC for 12 months are given in Tables 68 and 69, respectively. As is commonly seen in older mice, amyloidosis was common, especially in the intestine and kidney. A variety of other lesions, usually degenerative or inflammatory, were seen. However, there was no consistent relationship between any of the lesions and the treatment.

2. Feeding for 24 Months

Average organ weights of mice fed NC for 24 months are given in Table 70. The only differences seen in heart weight of the low dose mice were normal variation, not due to the feeding of NC.

Tissue lesions from mice fed the control or high (10% NC) dose and killed after 24 months feeding or dying at unscheduled times are shown in Tables 71 through 75. Tumor incidence is summarized in Table 76. These mice had a variety of lesions, as might be expected in geriatric mice. Amyloid deposits, often heavy, were widespread. Many other lesions, mostly degenerative, were found in various tissues. There was a treatment-related difference in the lack of bronchoalveolar carcinomas in the high dose mice. The data are statistically significant ($p = 0.004$ for males, $p = 0.29$ for females, $p = 0.002$ for the combined sexes, by exact analysis of the contingency table). The difference probably represents natural variation and is toxicologically meaningless. Because of the lack of treatment-related effects, the slides from other dosage groups were not examined.

Organ weights from mice fed NC for 24 months and allowed to recover for 1 month are given in Table 77. As in mice not allowed to recover, there were no treatment-related differences. The slides were not examined.

F. Discussion and Conclusions

As with the rats, feeding of fiber, either nitrocellulose or cotton linters, caused increased feed consumption and, at 10% levels, decreased body weights. These changes are consistent with the fibers being inert bulk in the diet. The difference in body weight decreased in older age. The calculation of actual fiber consumption was unreliable because of scattering. There were no treatment-related effects on clinical laboratory data, on organ weight, or on histopathology.

As seen earlier,[6/] some mice could not handle the fibers, and died of intestinal impaction in the first 3 weeks of study. This was a purely mechanical effect. The hyperemia, or "red ear syndrome," is probably either a coincidence or an irritation reaction due to the fiber.

The cause of the cluster of deaths about Month 9 in the high dose and cotton control mice is unknown. There was no obvious epidemiology. The lack of obvious moribundicity, followed by the rapid autolysis characteristic of mice, precluded histopathological elucidation. The cluster of deaths occurred in mice fed both fibers, so it could be due to some undefined fiber effect. However, there were three times more deaths among the NC mice than among the cotton control mice. The presence of a compound effect cannot be dismissed.

In conclusion, a diet of 10% fiber had adverse effects causing early deaths due to intestinal impaction and deaths after 9 months' feeding through an unknown mechanism in mice. Feeding 10% NC might be more hazardous than 10% linters. A diet of 3% NC had no effect, except that an increase in feed consumption was related to the inert bulk of the fiber.

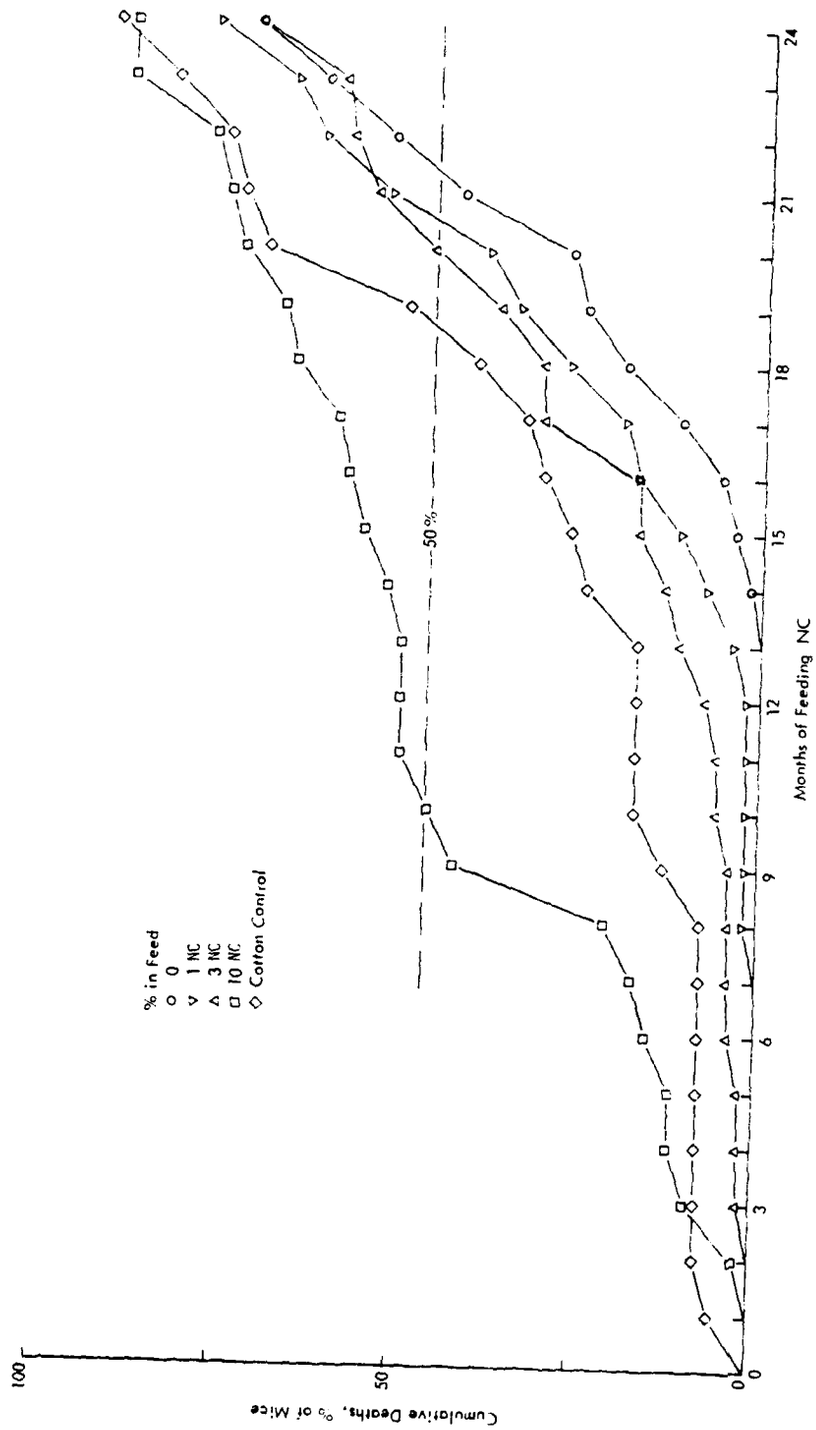

Figure 9 - Cumulative Unscheduled Deaths in Male Mice Fed NC

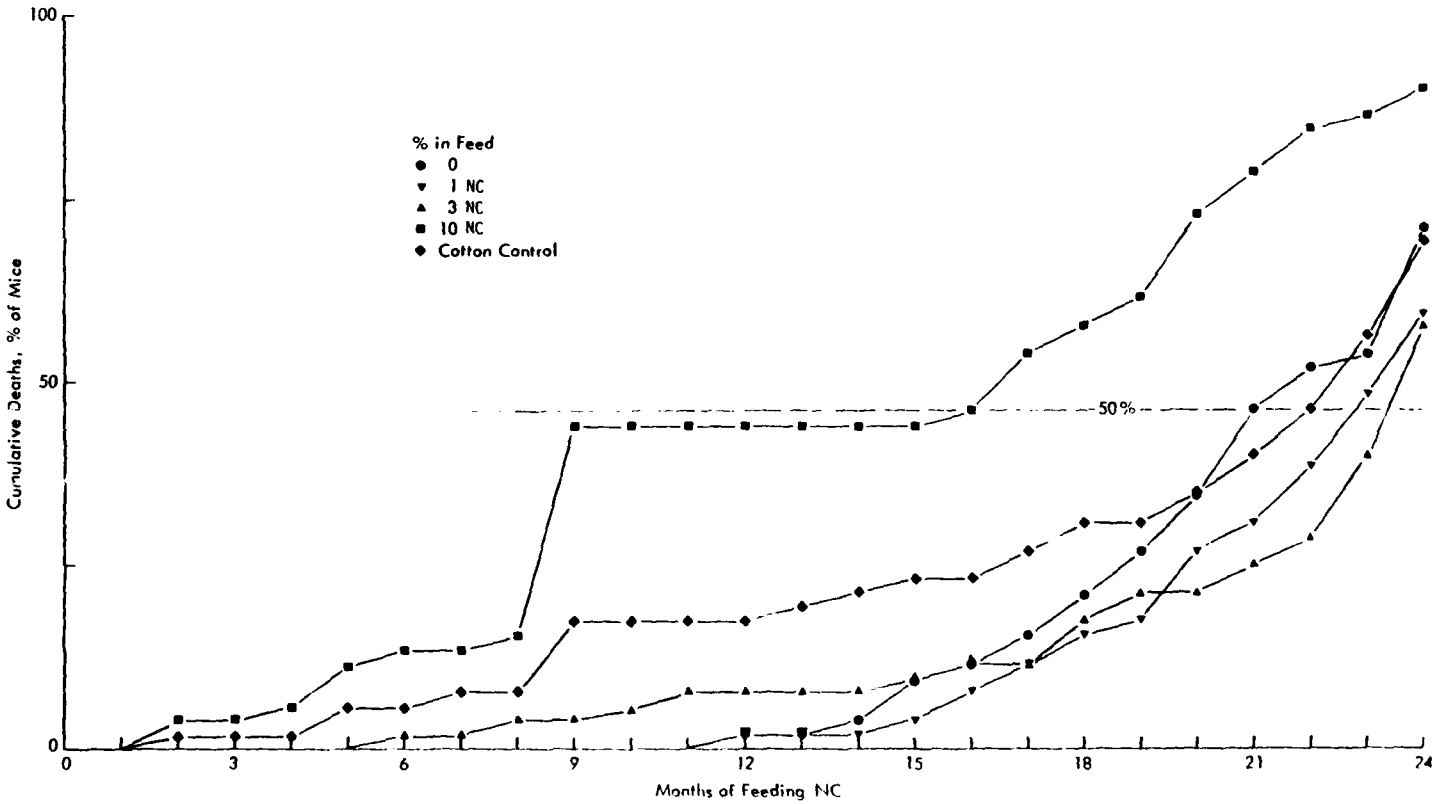

Figure 10 - Cumulative Unscheduled Deaths in Female Mice Fed NC

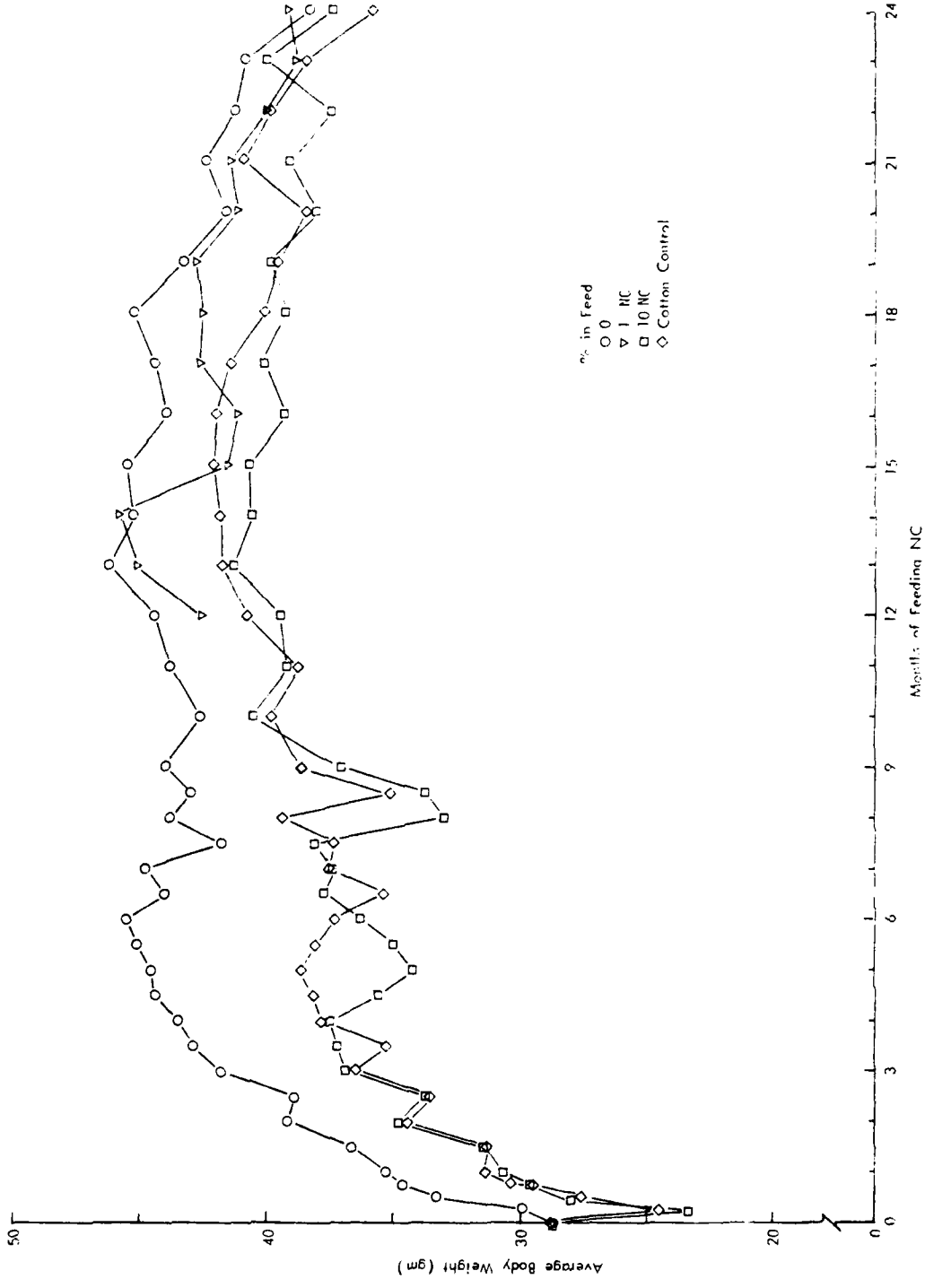

Figure 11 - Average Body Weights of Male Mice Fed NC

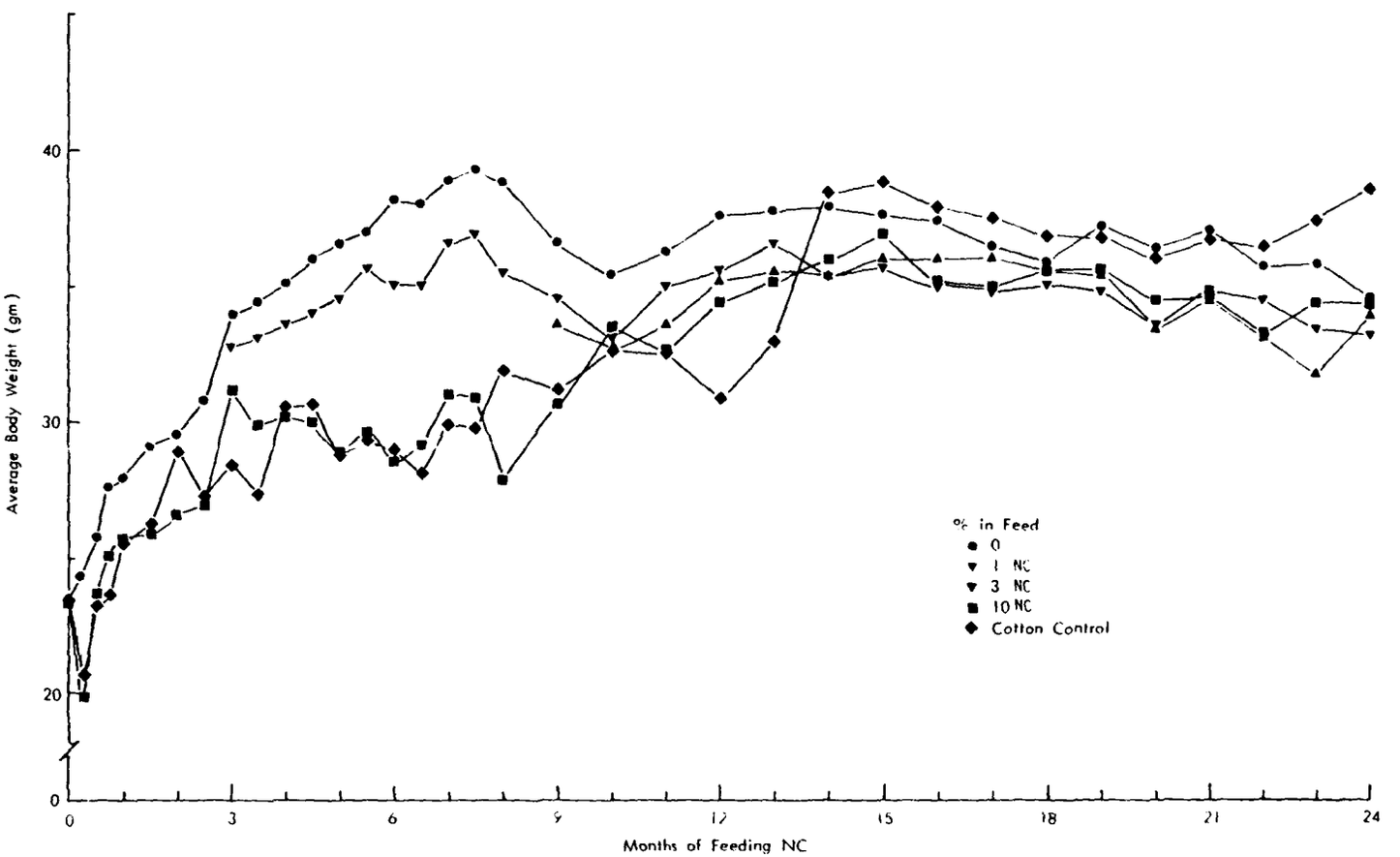

Figure 12 - Average Body Weights of Female Mice Fed NC

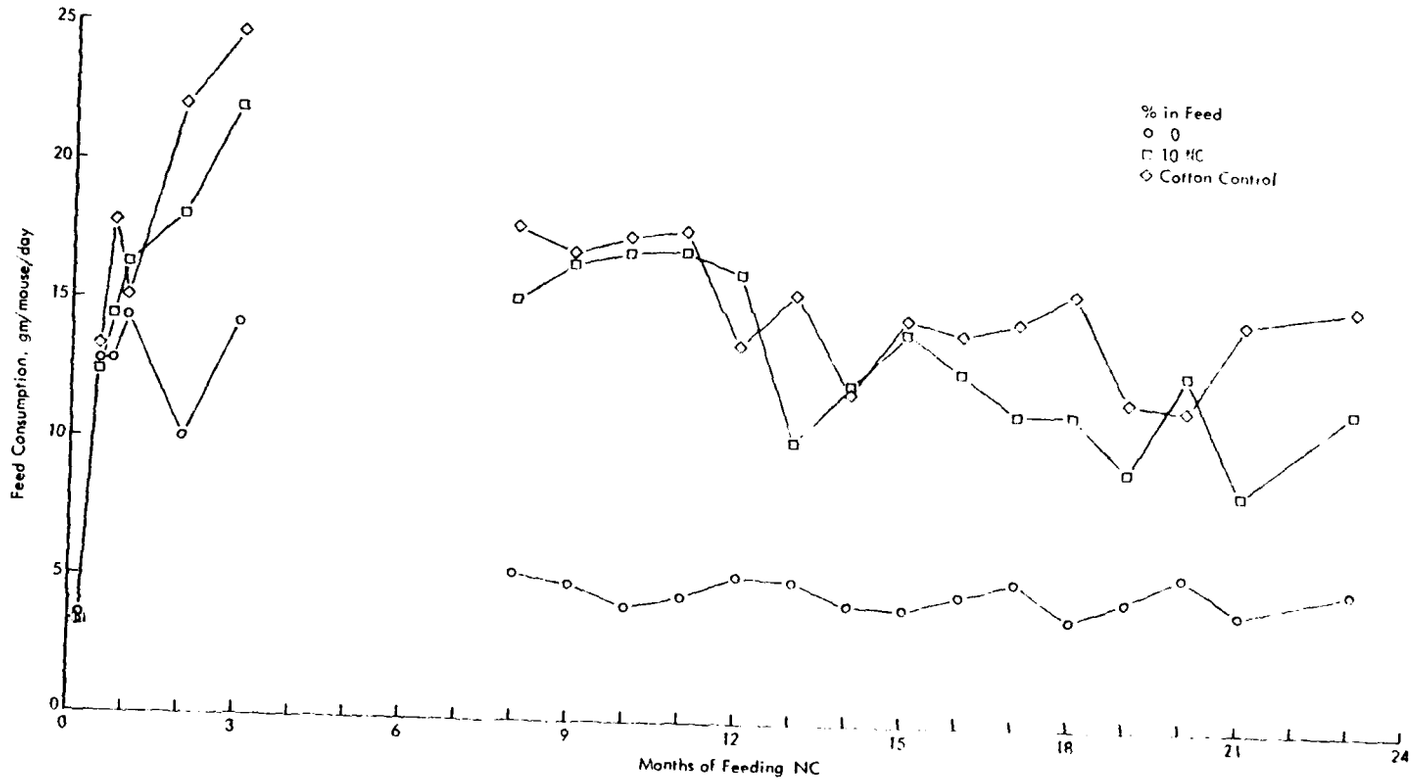

Figure 13 - Apparent Feed Consumption of Male Mice Fed NC

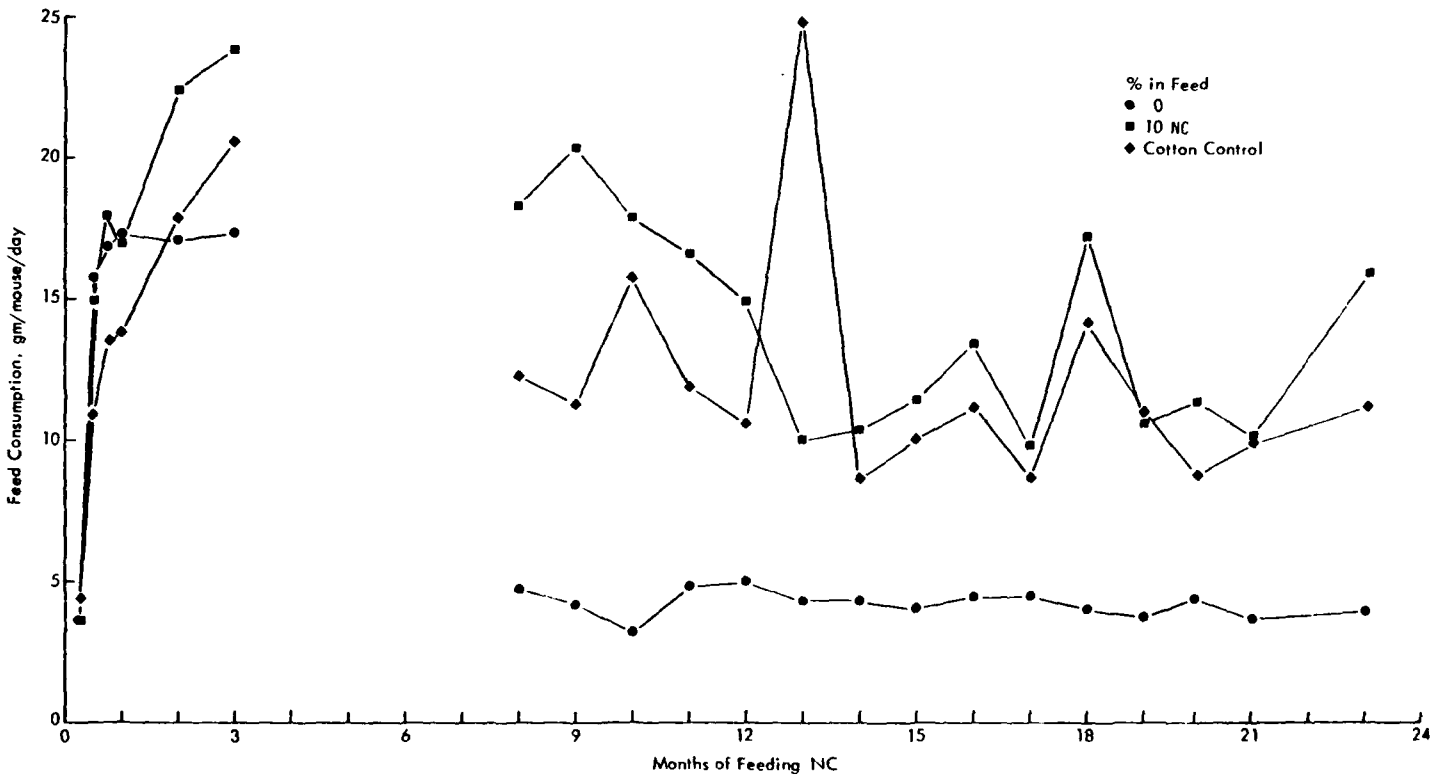

Figure 14 - Apparent Feed Consumption of Female Mice Fed NC

TABLE 59

APPARENT FEED CONSUMPTION OF MICE FED NC FOR 24 MONTHS

Dose % in Feed	Apparent Feed Consumption (g/mouse/day) Males	Females
0	5.91 ± 0.66[b/]	6.16 ± 1.08
1	6.53 ± 0.84	6.36 ± 1.21
3	6.65 ± 0.85	6.46 ± 1.10
10	13.82 ± 0.83[c/]	14.89 ± 1.05[c/]
10C[a/]	15.78 ± 0.81[c/]	12.76 ± 1.04

a/ Fed 10% cotton linters.

b/ Mean ± standard error of 18 measurements; the first month is the average of four measurements.

c/ Significantly different from control by Dunnett's multiple comparison procedures.

TABLE 60

LABORATORY DATA OF MICE FED NC AND DYING AT UNSCHEDULED TIMES

Dose (% in feed):	10C[a]	3	0	10C	3	3	10C	10C	0
Mouse Number:	50-041	53-732	51-317	50-120	53-654	53-656	50-020	50-024	51-214
Week of Death:	43	44	49	53	53	53	59	59	79
Erythrocytes, x 10^6/mm^3	6.35	1.78	3.79	2.27	3.15	4.40	6.41	6.44	4.08
Heinz bodies, %	0.00	0.00	-	-	-	-	0.00	0.15	1.07
Reticulocytes, %	2.82	0.15	10.49	15.1	5.31	3.26	1.55	1.90	0.00
Hematocrit, vol. %	42	13	27	24	31	35	38	48	20
Hemoglobin, g %	12.7	4.2	7.8	7.4	9.7	11.1	11.8	12.8	6.8
Methemoglobin, %	0.0	0.0	-	-	-	-	4.2	5.5	0.0
MCV, cubic microns	66.1	73.0	71.2	105.7	98.4	79.5	59.3	74.5	79.0
MCHB, picograms	20.0	23.6	20.6	32.6	30.8	25.2	18.4	19.9	16.7
MCHBC, g %	30.2	32.3	28.9	30.8	31.3	31.7	31.1	26.7	34.0
Platelets, x 10^5/mm^3	5.80	2.85	2.20	3.15	-	-	4.50	6.65	0.80
Leucocytes, x 10^3/mm^3	4.6	2.0	9.5	9.2	9.0	3.3	3.0	3.2	1.7
Neutrophils, %	48	78	19	11	34	27	33	17	26
Lymphocytes, %	51	22	80	88	64	73	67	83	54
Bands, %	0	0	0	0	0	0	0	0	18[b]
Monocytes, %	0	0	0	1	0	0	0	0	2
Eosinophils, %	1	0	1	0	2	0	0	0	0
Basophils, %	0	0	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0	0	1
SGPT, IU/liter	37	31	-	-	34	-	195	167	74
BUN, mg %	32	41	-	-	31	24	56	25	176

(Continued)

TABLE 60 (continued)

Dose (% in feed):	0	10	1	10	10	10C	10C	10	10C	10C
Mouse Number:	51-332	54-812	52-521	54-818	54-922	50-130	50-010	54-951	50-142	50-149
Week of Death:	81	81	84	85	85	85	89	89	90	90
Erythrocytes, x 10^6/mm^3	5.91	4.69	7.33	6.60	5.96	2.28	4.76	7.91	6.17	6.80
Heinz bodies, %	-	-	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00
Reticulocytes, %	2.86	3.90	1.89	4.14	0.29	25.00	2.50	0.66	0.93	1.15
Hematocrit, vol. %	37	38	38	34	35	-	30	42	39	42
Hemoglobin, g %	11.3	11.4	12.3	11.3	11.5	6.4	10.2	13.0	11.8	12.9
Methemoglobin, %	-	-	2.0	4.4	0.0	-	1.9	0.0	0.0	0.0
MCV, cubic microns	62.6	81.0	51.8	51.5	58.7	-	63.0	53.1	63.2	61.8
MCHB, picograms	19.1	24.3	16.8	17.1	19.3	28.1	21.4	16.4	19.1	19.0
MCHBC, g %	30.5	30.0	32.4	33.2	32.9	-	34.0	31.0	30.3	30.7
Platelets, x 10^5/mm^3	4.00	4.40	2.00	3.50	2.80	-	5.20	0	7.15	6.50
Leucocytes, x 10^3/mm^3	29.0	91.7	4.3	7.0	1.3	15.5	5.2	5.8	3.1	0.4
Neutrophils, %	70	65	24	27	37	44	42	18	40	42
Lymphocytes, %	30	16	76	72	63	56	55	80	60	58
Bands, %	0	15[c/]	0	0	0	0	0	0	0	0
Monocytes, %	0	4	0	0	0	0	0	1	0	0
Eosinophils, %	0	0	0	1	0	0	0	1	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0	0	0	0
SGPT, IU/liter	71	46	74	52	-	-	-	24	28	34
BUN, mg %	80	25	44	37	-	-	-	22	34	38

TABLE 60 (continued)

Dose (% in feed):	10C	10C	10C	10C	10	0	10	10	10C	
Mouse Number:	50-126	50-127	50-128	50-159	54-835	51-221	54-823	54-830	50-116	54-928
Week of Death:	94	94	94	96	96	97	97	97	102	102
Erythrocytes, x 10^6/mm^3	3.37	5.07	4.70	5.40	4.86	3.75	6.64	8.38	4.78	2.90
Heinz bodies, %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reticulocytes, %	1.54	1.03	1.99	3.67	0.77	1.43	0.76	1.35	0.52	9.78
Hematocrit, vol. %	25	37	41	34	27	18	33	44	26	18
Hemoglobin, g %	7.6	11.3	12.4	10.4	8.6	6.7	11.5	14.7	8.4	5.9
Methemoglobin, %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	3.4
MCV, cubic microns	74.2	73.0	87.2	63.0	55.6	48.0	49.7	52.5	54.4	62.1
MCHB, picograms	22.6	22.3	26.4	19.3	17.7	17.9	17.3	17.5	17.6	20.3
MCHBC, g %	30.4	30.5	30.2	30.6	31.9	37.2	34.8	33.4	32.3	32.8
Platelets, x 10^5/mm^3	5.50	6.50	7.95	3.10	1.60	2.20	2.55	3.00	2.95	2.70
Leucocytes, x 10^3/mm^3	12.0	8.0	7.2	12.3	2.1	3.2	4.0	4.5	4.2	2.5
Neutrophils, %	73	52	26	65	60	82	70	66	19	72
Lymphocytes, %	23	45	74	35	40	18	30	34	80	24
Bands, %	4	2	0	0	0	0	0	0	0	4
Monocytes, %	0	0	0	0	0	0	0	0	1	0
Eosinophils, %	0	1	0	0	0	0	0	0	0	0
Basophils, %	0	0	0	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0	0	0	0
Nucleated erythrocytes, %	1	0	0	0	0	0	0	0	0	0
SGPT, IU/liter	28	55	114	40	-	28	37	40	46	28
BUN, mg %	55	32	35	48	60	218	94	-	63	180

TABLE 60 (concluded)

Dose (% in feed):	10C	10C	0	0	1	10C	0
Mouse Number:	50-113	50-017	51-325	51-326	52-438	50-114	51-314
Week of Death:	102	103	103	103	103	104	108[e]
Erythrocytes, x 10^6/mm^3	6.26	7.07	4.75	1.17	8.19	1.63	4.24
Heinz bodies, %	-	0.00	0.00	0.00	0.00	0.00	0.00
Reticulocytes, %	5.63	2.11	12.30	7.89	1.15	4.50	6.19
Hematocrit, vol. %	41	40	32	-	42	11	24
Hemoglobin, g %	13.5	13.5	11.0	3.5	15.8	3.9	8.3
Methemoglobin, %	-	0.0	4.6	0.0	0.0	0.0	6.0
MCV, cubic microns	65.5	56.6	67.4	-	51.3	67.5	56.6
MCHB, picograms	21.6	19.1	23.2	29.9	19.3	23.9	19.6
MCHBC, g %	32.9	33.8	34.4	-	37.6	35.5	34.6
Platelets, x 10^5/mm^3	-	6.75	4.10	-	2.10	2.00	3.75
Leucocytes, x 10^3/mm^3	61.7	3.8	16.6	13.6	1.2	5.0	7.0
Neutrophils, %	32	38	34	31	40	17	52
Lymphocytes, %	31	61	66	69	60	83	48
Bands, %	20[d]	0	0	0	0	0	0
Monocytes, %	3	0	0	0	0	0	0
Eosinophils, %	14	1	0	0	0	0	0
Basophils, %	0	0	0	0	0	0	0
Atypical, %	0	0	0	0	0	0	0
Nucleated erythrocytes, %	0	0	0	0	0	0	0
SGPT, IU/liter	-	34	24	-	-	31	-
BUN, mg %	-	27	25	-	168	91	81

a/ Fed 10% cotton linters.
b/ 14% bands, 4% metamyelocytes.
c/ 5% bands, 5% metamyelocytes, 5% myelocytes.
d/ 12% bands, 5% metamyelocytes, 2% myelocytes, 1% myeloblasts.
e/ Died in week 4 of recovery after 24 months feeding.

TABLE 61

LABORATORY DATA OF MALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 13 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0.00 (C. 4)	1.00 (T. 4)	3.00 (T. 4)	10.00 (T. 4)	10[a] (C. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	5.68 ± .15	4.76 ± 1.38	6.21 ± .21 (3)	6.92 ± .29	7.01 ± .12
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.29 ± .12	1.34 ± .28	.96 ± .21	1.28 ± .13	.78 ± .11
HEMATOCRIT, VOL. %	41.0 ± .7	40.3 ± 2.5	43.3 ± .3 (3)	44.0 ± 1.5	44.3 ± .3
HEMOGLOBIN, GM. %	12.4 ± .2	11.8 ± .9	12.1 ± .2 (3)	13.5 ± .6	13.4 ± .1
METHEMOGLOBIN, %	.4 ± .4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	72.3 ± 2.3	187.4 ±117.9	69.9 ± 2.3 (3)	63.7 ± .6	63.2 ± 1.2
MCHB, MICRO MICROGMS.	22.0 ± .9	52.9 ± 32.3	19.6 ± .3 (3)	19.5 ± .2	19.1 ± .3
MCHBC, GM %	30.4 ± .4	29.2 ± .6	28.0 ± .6 (3)[b]	30.7 ± .5	30.3 ± .4
PLATELETS ($X10^5$ /MM^3)	4.7 ± 1.0 (2)	5.0 ± .2	5.8 ± .3	5.8 ± .5	5.7 ± .9
LEUKOCYTES ($X10^3$ /MM^3)	4.0 ± .4	4.4 ± .8	5.2 ± 1.0 (3)	10.2 ± 4.0	3.5 ± .7
NEUTROPHILS, %	26.0 ± 1.4	24.0 ± 4.9	19.5 ± 2.1	18.8 ± 6.2	17.5 ± 2.6
LYMPHOCYTES, %	71.3 ± .8	75.0 ± 5.2	78.8 ± 2.6	79.3 ± 6.4	79.8 ± 3.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	.8 ± .5	.8 ± .3	1.5 ± .6	.8 ± .5	2.0 ± .4
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	2.0 ± 1.2	.3 ± .3	.3 ± .3	1.3 ± .8	.8 ± .3
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	26.3 ± 9.9 (3)	24.3 ± 8.3	21.3 ± 4.8 (3)	64.5 ± 16.7	54.8 ± 6.3
BUN, MG %	33.5 ± 3.2	30.3 ± 3.2	22.0 ± 1.5 (3)[b]	22.0 ± .4[b]	26.5 ± .9

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 62

LABORATORY DATA OF FEMALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 12 MONTHS

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0.00 (C. 4)	1.00 (T. 4)	3.00 (T. 4)	10.00 (T. 4)	100 a/ (T. 4)
ERYTHROCYTES ($X10^6$ /MM^3)	6.38 ± .11	6.28 ± .25	6.56 ± .40 (3)	7.79 ± .02 b/	6.57 ± .31
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	.80 ± .09	.76 ± .11	.88 ± .15 (3)	1.09 ± .07	1.25 ± .41
HEMATOCRIT, VOL. %	43.8 ± 1.0	45.3 ± 1.1	44.3 ± 2.2 (3)	46.3 ± .5	43.0 ± 1.5
HEMOGLOBIN, GM. %	13.2 ± .3	13.0 ± .5	13.1 ± 1.0 (3)	14.5 ± .1	12.8 ± .8
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	68.6 ± 1.3	72.2 ± 1.1	67.7 ± 1.2 (3)	59.4 ± .7 b/	65.7 ± 2.4
MCHB, MICRO MICROGMS.	20.8 ± .3	20.8 ± .2	20.0 ± .2 (3)	18.6 ± .1 b/	19.5 ± .4 b/
MCHBC, GM %	30.3 ± .3	28.8 ± .5	29.6 ± .9 (3)	31.4 ± .3	29.8 ± 1.2
PLATELETS ($X10^5$ /MM^3)	4.2 ± .5	4.5 ± .8	4.3 ± .7 (3)	5.6 ± .8	6.0 ± .6
LEUKOCYTES ($X10^3$ /MM^3)	4.7 ± .4	5.2 ± .8	6.3 ± 1.4 (3)	5.6 ± 1.1	4.7 ± .9
NEUTROPHILS, %	25.3 ± 4.3	23.0 ± 4.0	19.3 ± 7.4 (3)	18.8 ± 3.9	30.5 ± .6
LYMPHOCYTES, %	71.0 ± 4.5	75.0 ± 3.9	73.7 ± 7.9 (3)	80.3 ± 3.8	67.0 ± 1.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	3.0 ± 1.0	1.0 ± .4	2.3 ± 1.4	.5 ± .3	1.8 ± 1.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.8 ± .3	1.0 ± .6	1.5 ± .9	.5 ± .3	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGPT, IU/L	26 ± 7	22 ± 4	29 ± 2 (3)	85 ± 28	121 ± 35 b/
BUN, MG %	23.3 ± 1.5 (3)	29.8 ± 4.7	23.7 ± 1.9 (3)	23.5 ± .5	27.5 ± 4.5

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 63

LABORATORY DATA OF MALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0 (C, 4)	1 (T, 4)	3 (T, 4)	10 (T, 3)	10[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.08 ± .94	6.92 ± .86	7.68 ± .36	8.14 ± .64	7.44 ± .27
HEINZ BODIES, %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
RETICULOCYTES, %	1.29 ± .12 (3)	1.41 ± .28 (3)	1.73 ± .52	2.30 ± .40	1.29 ± .17
HEMATOCRIT, VOL. %	38.8 ± 5.2	39.0 ± 4.9	42.3 ± 1.7	45.7 ± 2.2	41.8 ± 1.3
HEMOGLOBIN, GM. %	13.6 ± 1.5	13.3 ± 1.2	14.3 ± .6	16.2 ± 1.2	13.9 ± .4
METHEMOGLOBIN, %	0.0 ± 0.0	.5 ± .5	0.0 ± 0.0	1.7 ± 1.1	0.0 ± 0.0
MCV, CUBIC MICRONS	54.6 ± .6	56.3 ± .9	55.1 ± .8	56.4 ± 1.8	56.2 ± 1.1
MCHB, MICRO MICROGMS.	19.5 ± .7	19.6 ± .9	18.7 ± .2	20.0 ± 1.6	18.7 ± .3
MCHBC, GM %	35.6 ± 1.3	34.8 ± 1.8	33.9 ± .4	35.5 ± 2.7	33.3 ± .3
PLATELETS ($X10^5$ /MM^3)	5.3 ± .8	4.4 ± .9	4.6 ± .5	4.3 ± .2	4.6 ± .3
LEUKOCYTES ($X10^3$ /MM^3)	2.7 ± .5	2.5 ± .9	2.6 ± 1.6	12.4 ± 8.0	3.0 ± .3
NEUTROPHILS, %	34.3 ± 10.3	41.5 ± 6.2	26.5 ± 8.2	28.0 ± 7.8	20.3 ± 5.0
LYMPHOCYTES, %	65.8 ± 10.3	58.0 ± 6.2	73.5 ± 8.2	71.0 ± 8.2	79.0 ± 5.5
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± .6	.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	.5 ± .5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	142 ± 89 (3)	106 ± 13 (3)	105 ± 10	145 ± 42	101 ± 21 (3)
SGPT, IU/L	75.3 ± 39.9 (3)	51.0 ± 11.8	33.8 ± 5.7	69.7 ± 39.3	37.3 ± 7.3
BUN, MG %	54.3 ± 19.3 (3)	43.3 ± 14.1 (3)	44.0 ± 11.0	31.3 ± 6.2	32.8 ± 2.5

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.

b/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 64

LABORATORY DATA OF FEMALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS

(C,N) CONTROL (T,N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0 (C, 4)	1 (T, 3)	3 (T, 5)	10 (T, 4)	10[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.05 ± .21	6.57 ± .97 (2)	5.99 ± 1.01	6.33 ± .21	8.20 ± .67
HEINZ BODIES, %	0.0 ± 0.0	.3 ± .3	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0
RETICULOCYTES, %	1.51 ± .53	4.07 ± .18 (2)[b/]	1.59 ± .28 (4)	1.62 ± .35	1.40 ± .14
HEMATOCRIT, VOL. %	41.0 ± .6 (3)	32.0 ± 7.0 (2)	39.5 ± 1.3 (4)	36.5 ± 1.4	43.5 ± 2.6
HEMOGLOBIN, GM. %	13.4 ± .4	11.4 ± .8	12.6 ± .4	12.0 ± .4	15.1 ± 1.2
METHEMOGLOBIN, %	0.0 ± 0.0	2.6 ± 1.5	.4 ± .4	2.2 ± .9	0.0 ± 0.0
MCV, CUBIC MICRONS	58.7 ± 1.9 (3)	57.0 ± 2.6 (2)	56.5 ± 1.2 (4)	57.6 ± 1.1	53.3 ± 1.2
MCHB, MICRO MICROGMS.	19.0 ± .3	20.5 ± 1.2 (2)	26.2 ± 7.8	19.0 ± .4	18.5 ± .2
MCHBC, GM %	32.3 ± 1.0 (3)	36.2 ± 3.8 (2)	32.6 ± .2 (4)	33.0 ± .3	34.7 ± .8
PLATELETS ($X10^5$ /MM^3)	5.4 ± .9	4.2 ± 1.1	3.8 ± .7	14.8 ± 9.6	4.6 ± .5
LEUKOCYTES ($X10^3$ /MM^3)	3.4 ± 1.2	4.4 ± 1.1	2.4 ± .6	3.6 ± 1.2	8.5 ± 2.5
NEUTROPHILS, %	30.5 ± 7.6	26.7 ± 13.4	25.8 ± 9.9	30.3 ± 4.8	19.5 ± 4.9
LYMPHOCYTES, %	69.5 ± 7.6	40.0 ± 20.1	54.0 ± 15.5	69.5 ± 4.7	79.0 ± 4.4
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.3 ± .3	.8 ± .5
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	.2 ± .2	0.0 ± 0.0	.8 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	123 ± 43 (3)	70 ± 54 (2)	91 ± 13 (4)	100 ± 15	163 ± 50
SGPT, IU/L	35.0 ± 5.6 (3)	40.0 ± 3.0 (2)	29.0 ± 3.8 (4)	29.0 ± 4.2	55.0 ± 13.5
BUN, MG %	31.3 ± 4.8 (3)	139.0 ± 86.0 (2)[b/]	38.8 ± 8.6 (4)	59.3 ± 15.7	30.0 ± 6.3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 65

LABORATORY DATA OF MALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS AND ALLOWING TO RECOVERY FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0 (C, 3)	1 (T, 3)	3 (T, 4)	10 (T, 2)
ERYTHROCYTES ($X10^6$ /MM^3)	7.59 ± .53	7.59 ± .49	8.06 ± .38	7.63 ± .41
HEINZ BODIES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.0 ± .0
RETICULOCYTES, %	1.59 ± .58	1.61 ± .31	.87 ± .21	1.17 ± .21
HEMATOCRIT, VOL. %	42.3 ± 1.7	42.0 ± 2.1	45.8 ± 2.3	43.5 ± 2.5
HEMOGLOBIN, GM. %	14.0 ± .4	13.8 ± .7	14.8 ± .6	13.9 ± .8
METHEMOGLOBIN, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	.9 ± .9
MCV, CUBIC MICRONS	56.0 ± 1.9	55.5 ± 1.5	56.8 ± .8	57.0 ± .2
MCHB, MICRO MICROGMS.	18.6 ± .9	18.2 ± .4	18.5 ± .5	18.3 ± .0
MCHBC, GM %	33.1 ± .5	32.9 ± .2	32.5 ± .6	32.1 ± .1
PLATELETS ($X10^5$ /MM^3)	6.4 ± 2.1	4.8 ± .2	4.5 ± .8	4.8 ± .2
LEUKOCYTES ($X10^3$ /MM^3)	3.0 ± 2.2	2.6 ± 1.1	2.8 ± .7	1.8 ± 0.0
NEUTROPHILS, %	31.3 ± 6.9	36.0 ± 9.2	31.3 ± 5.6	40.0 ± 8.0
LYMPHOCYTES, %	68.0 ± 6.7	63.0 ± 9.3	68.3 ± 5.4	60.0 ± 8.0
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	.3 ± .3	.5 ± .5	0.0 ± 0.0
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MONOCYTES, %	.7 ± .7	.7 ± .7	0.0 ± 0.0	0.0 ± 0.0
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SGOT, IU/L	141 ± 44	122 ± 18	144 ± 12	150 ± 64
SGPT, IU/L	22.3 ± 5.5	60.7 ± 12.4	67.8 ± 14.8	52.5 ± 18.5
BUN, MG %	59.0 ± 23.5	40.0 ± 10.8	56.0 ± 18.5	47.0 ± 17.0

ENTRIES ARE MEAN ± STANDARD ERROR

a/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 66

LABORATORY DATA OF FEMALE MICE AFTER FEEDING OF NITROCELLULOSE FOR 24 MONTHS AND ALLOWING TO RECOVERY FOR 1 MONTH

(C.N) CONTROL (T.N) TREATED N = NUMBER OF MICE

DOSE: % IN FEED	0 (C, 3)	1 (T, 3)	3 (T, 3)	10 (T, 1)	10[a/] (C, 4)
ERYTHROCYTES ($X10^6$ /MM^3)	7.13 ± .49	7.03 ± .20	7.23 ± .10	6.50	7.45 ± .18
HEINZ BODIES, %	.0 ± .0	.0 ± .0	0.0 ± 0.0	0.0	0.0 ± 0.0
RETICULOCYTES, %	1.26 ± .52	1.33 ± .48	.90 ± .12	1.14	1.02 ± .15
HEMATOCRIT, VOL. %	40.3 ± 1.5	40.0 ± .6	42.0 ± 1.5	37.0	43.3 ± .9
HEMOGLOBIN, GM. %	13.1 ± .5	13.1 ± .3	13.8 ± .6	12.3	14.2 ± .2
METHEMOGLOBIN, %	0.0 ± 0.0	2.4 ± 2.4	0.0 ± 0.0	0.0	0.0 ± 0.0
MCV, CUBIC MICRONS	56.8 ± 1.8	56.9 ± .9	58.0 ± 1.3	56.9	58.1 ± 1.4
MCHB, MICRO MICROGMS.	18.5 ± .5	18.7 ± .3	19.1 ± .6	18.9	19.0 ± .3
MCHBC, GM %	32.6 ± .2	32.8 ± .6	32.9 ± .3	33.2	32.8 ± .4
PLATELETS ($X10^5$ /MM^3)	4.9 ± .2	4.6 ± .9	4.0 ± .4	6.4	4.2 ± .7
LEUKOCYTES ($X10^3$ /MM^3)	2.4 ± .9	1.9 ± .6	1.6 ± .4	1.3	2.3 ± .7
NEUTROPHILS, %	34.7 ± 13.8	32.3 ± 4.9	28.0 ± 6.7	28.0	25.3 ± 1.8
LYMPHOCYTES, %	65.3 ± 13.8	67.7 ± 4.9	72.0 ± 6.7	72.0	73.5 ± 2.8
BANDS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
EOSINOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	.8 ± .8
BASOPHILS, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
MONOCYTES, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	.5 ± .5
ATYPICAL, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
NUCLEATED RBC, %	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	0.0 ± 0.0
SGOT, IU/L	170 ± 38	218 ± 114	146 ± 62	142	117 ± 12
SGPT, IU/L	76.0 ± 24.1	79.3 ± 49.8	92.3 ± 54.0	31.0	40.3 ± 6.6
BUN, MG %	33.7 ± 4.7	36.0 ± 2.6	48.0 ± 14.6	90.0	27.5 ± 1.3

ENTRIES ARE MEAN ± STANDARD ERROR

a/ FED 10% COTTON LINTERS.
b/ SIGNIFICANTLY DIFFERENT FROM CONTROL MICE BY DUNNETT'S MULTIPLE COMPARISON PROCEDURE.

TABLE 67

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED NC FOR 12 MONTHS

Sex	Dose (% in feed)	Terminal Body Weight (g)	Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
			Absolute Organ Weight (g)						
Male	0	42 ± 2[b]	0.46 ± 0.01	0.20 ± 0.01	1.78 ± 0.24	0.63 ± 0.04	0.13 ± 0.01	0.24 ± 0.02	
	1	42 ± 3[b]	0.50 ± 0.03	0.19 ± 0.01	1.57 ± 0.15	0.65 ± 0.02	0.13 ± 0.02	0.28 ± 0.04	
	3	45 ± 2[b]	0.50 ± 0.01	0.20 ± 0.01	1.48 ± 0.10	0.65 ± 0.04	0.12 ± 0.02	0.29 ± 0.01	
	10	41 ± 1[b]	0.46 ± 0.02	0.21 ± 0.02	1.53 ± 0.07	0.63 ± 0.02	0.15 ± 0.06	0.29 ± 0.01	
	10C[a]	45 ± 3[b]	0.47 ± 0.02	0.21 ± 0.01	1.64 ± 0.09	0.70 ± 0.00	0.13 ± 0.01	0.25 ± 0.04	
Female	0	35 ± 3[b]	0.49 ± 0.01	0.15 ± 0.01	1.31 ± 0.07	0.42 ± 0.02	0.10 ± 0.01		0.023 ± 0.003
	1	37 ± 1[b]	0.50 ± 0.01	0.15 ± 0.01	1.45 ± 0.07	0.45 ± 0.04	0.13 ± 0.01		0.038 ± 0.011
	3	36 ± 2[c]	0.48 ± 0.03	0.14 ± 0.01	1.36 ± 0.09	0.43 ± 0.03	0.10 ± 0.01		0.050 ± 0.001
	10	38 ± 1[b]	0.49 ± 0.01	0.15 ± 0.01	1.27 ± 0.06	0.43 ± 0.02	0.14 ± 0.01		0.049 ± 0.009
	10C	34 ± 2[b]	0.49 ± 0.01	0.17 ± 0.02	1.37 ± 0.11	0.44 ± 0.02	0.19 ± 0.03[d]		0.085 ± 0.026[d]
			Relative Organ Weight (g/100 g body weight)						
Male	0		1.11 ± 0.07	0.47 ± 0.03	4.18 ± 0.45	1.51 ± 0.10	0.32 ± 0.03	0.58 ± 0.07	
	1		1.22 ± 0.11	0.45 ± 0.02	3.76 ± 0.27	1.56 ± 0.07	0.32 ± 0.04	0.66 ± 0.07	
	3		1.13 ± 0.05	0.45 ± 0.03	3.31 ± 0.07	1.46 ± 0.11	0.28 ± 0.05	0.65 ± 0.05	
	10		1.11 ± 0.04	0.50 ± 0.03	3.74 ± 0.23	1.54 ± 0.03	0.39 ± 0.16	0.71 ± 0.03	
	10C		1.09 ± 0.13	0.49 ± 0.05	3.69 ± 0.07	1.61 ± 0.11	0.30 ± 0.04	0.60 ± 0.13	
Female	0		1.47 ± 0.15	0.42 ± 0.02	3.85 ± 0.17	1.24 ± 0.11	0.28 ± 0.01		0.067 ± 0.007
	1		1.36 ± 0.03	0.39 ± 0.02	3.95 ± 0.11	1.24 ± 0.12	0.35 ± 0.03		0.102 ± 0.026
	3		1.34 ± 0.03	0.39 ± 0.02	3.78 ± 0.22	1.19 ± 0.04	0.29 ± 0.01		0.139 ± 0.004
	10		1.31 ± 0.08	0.41 ± 0.02	3.38 ± 0.08	1.17 ± 0.09	0.37 ± 0.02		0.130 ± 0.024
	10C		1.46 ± 0.10	0.52 ± 0.06	4.05 ± 0.14	1.32 ± 0.11	0.55 ± 0.06[d]		0.247 ± 0.070[d]
			Relative Organ Weight (g/g brain weight)						
Male	0			0.43 ± 0.04	3.88 ± 0.60	1.38 ± 0.11	0.29 ± 0.03	0.52 ± 0.04	
	1			0.37 ± 0.04	3.15 ± 0.32	1.30 ± 0.07	0.26 ± 0.03	0.55 ± 0.07	
	3			0.40 ± 0.04	2.94 ± 0.16	1.30 ± 0.11	0.25 ± 0.05	0.58 ± 0.04	
	10			0.45 ± 0.04	3.39 ± 0.27	1.39 ± 0.07	0.36 ± 0.15	0.64 ± 0.03	
	10C			0.45 ± 0.08	3.51 ± 0.33	1.50 ± 0.07	0.28 ± 0.02	0.54 ± 0.07	
Female	0			0.30 ± 0.02	2.67 ± 0.17	0.85 ± 0.05	0.19 ± 0.01		0.047 ± 0.007
	1			0.29 ± 0.02	2.91 ± 0.12	0.91 ± 0.09	0.26 ± 0.03		0.076 ± 0.021
	3			0.29 ± 0.01	2.82 ± 0.11	0.89 ± 0.01	0.21 ± 0.01		0.104 ± 0.004
	10			0.31 ± 0.01	2.61 ± 0.18	0.89 ± 0.04	0.28 ± 0.01		0.101 ± 0.018
	10C			0.36 ± 0.03	2.82 ± 0.23	0.91 ± 0.04	0.39 ± 0.06[d]		0.177 ± 0.056[d]

a/ Fed 10% cotton linters.
b/ Mean ± standard error of four mice.
c/ Mean ± standard error of three mice.
d/ Significantly different from control by Dunnett's multiple comparison procedure.

TABLE 68

SUMMARY OF LESIONS OF MALE MICE FED NC FOR 12 MONTHS

Dose (% of feed):	0				1				3				10				10C[a]			
Mouse Number:	201	202	203	204	401	402	403	404	601	602	603	604	803	804	805	806	001	002	003	004
Lesions[b]																				
Adrenal Gland																				
Amyloidosis			3			1	1	1									2			
Fibroblast proliferation	2	2	1	1	1		1	1	1		1		1	1	1		1			2
Ceroid degeneration	2	2		1	1	1		1	1	1	1	1		1	1	1	1	1	1	1
Thyroid																				
Amyloidosis			1																	
Thyroiditis											1									
Lung																				
Peribronchiolar lymphoid hyperplasia			1				1	1	1		1	1		1		1		1		
Focal fibrosis																		1		
Heart																				
Myocardial degeneration (amyloidosis)	1	1	1		1	1	1	1										1		
Liver																				
Polyploid hepatocytes	2	1		1					1						2	1	2	1	1	1
Inclusion body						1								1	2	1	1	1	1	
Portal inflammation		1				1	1	1							2			1	1	1
Focal necrosis															1					
Hepatoma	X		X																	
Amyloidosis			1			1	1													
Spleen																				
Excessive hematopoiesis			1												1					
Amyloidosis		2				2														
Lymph Node																				
Amyloidosis		1		1			1						1		1					
Salivary Gland																				
Foci of mononuclear cells					1	1				1	1			1		1	1			1
Intestine																				
Amyloidosis	3	3	4	3	3	3	4	4		1			3	2	3	4	3	1		
Parasitism	1		1		1	1	1	1					3				1	1	1	3
Lymphoid hyperplasia													1				1			
Kidney																				
Amyloidosis	1	1	4	1		1	2	2						1	1		3	1		
Perivascular lymphoid cuffs	1	1	4	1	1	1	2	1	1	1	1	1	1		1	1	2	1	1	1
Hydronephrosis			1					2												
Urinary Bladder																				
Foci of mononuclear cells														1	1					
Eye																				
Optic perineuritis																		1		
Retinal atrophy			1				2													
Testis																				
Amyloidosis			3		1															
Degeneration and/or atrophy			3		1									2						
Prostate																				
Focal mononuclear cells infiltration															1					
Bone Marrow																				
M/E ratio	1.7	1.4	2.3	1.6	1.2	0	2.1	2.1	1.0	1.3	0	0	1.2	1.0	4.0	1.6	2.0	3.2	1.3	1.4

Organs not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; + = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 69

SUMMARY OF LESIONS OF FEMALE MICE FED NC FOR 12 MONTHS

Dose (% of feed):	0				1				3			10				10C[a]			
Mouse No.:	301	302	303	304	501	502	503	504	702	703	704	902	905	906	907	101	106	107	108
Lesions[b]																			
Adrenal Gland																			
Amyloidosis							1	1					1		1	1		1	1
Fibroblast proliferation		1	1		1	1	1	1		1	1	1	1	1	2	1	1	1	1
Ceroid degeneration									1			1	1						
Thyroid																			
Amyloidosis							1	1											
Thyroiditis											1								
Lung																			
Peribronchiolar lymphoid hyperplasia	1				1	2	1	1	1	1	1				1		1		1
Focal fibrosis			1	1															
Heart																			
Myocardial degeneration (amyloidosis)							1	1											1
Liver																			
Polyploid hepatocytes		1	1	3				1				2							
Portal inflammation	1	1				2		1			1	3	1					1	3
Focal necrosis		1	1									1	1	1				1	3
Amyloidosis							1	1											1
Spleen																			
Excessive hematopoiesis														1		1			
Ovary																			
Amyloidosis					1	1	4	4					1	1				4	4
Ovarian cyst								1		1					1				
Uterus																			
Cystic hyperplasia of endometrium							2					4	1	1		1	1		
Endometritis								1	1										
Lymph Node																			
Amyloidosis								1											
Salivary Gland																			
Foci of mononuclear cells				1		1		1											
Stomach																			
Focal gastritis										1	1					1			
Intestine																			
Amyloidosis					3	2	4	4	3	3			3	3		3	3	4	4
Parasitism	1	1											1			1			
Lymphoid hyperplasia							1	1			1						1	1	
Kidney																			
Amyloidosis	1	1		1	1		2	2				1	1	1		1	1	2	3
Perivascular lymphoid cuffs	1	1		2		1	1	1			1			1	1	1	1	1	2
Nephrosis (tubular)				1															
Urinary Bladder																			
Foci of mononuclear cells						1		1		1	1						1		
Eye																			
Retinal atrophy		1	1			2											1	1	
Chorioditis																			1
Parathyroid																			
Amyloidosis							1												
Bone Marrow																			
M/E ratio	1.7	2.2	1.5	0	0	1.1	1.7	1.0	1.2	0	1.2	1.4	1.3	1.4	1.4	2.2	1.8	2.3	1.8

Organs not listed were normal.

a/ Fed 10% cotton linters.

b/ Severity of lesions: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; * = questionable; X = present; 0 = tissue missing or unreadable.

TABLE 70

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED 3C FOR 24 MONTHS

Sex	Dose (% in Feed)	Terminal Body Weight (g)	Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
			Absolute Organ Weight (g)						
Male	0	40 ± 2[b]	0.47 ± 0.02	0.22 ± 0.01	1.94 ± 0.13	0.70 ± 0.08	0.21 ± 0.02	0.21 ± 0.01	
	1	36 ± 4[c]	0.46 ± 0.02	0.24 ± 0.02	1.61 ± 0.19	0.63 ± 0.07	0.15 ± 0.03	0.19 ± 0.02	
	3	41 ± 3[c]	0.45 ± 0.01	0.21 ± 0.02	1.87 ± 0.11	0.71 ± 0.07	0.14 ± 0.02	0.22 ± 0.03	
	10	38 ± 2[d]	0.45 ± 0.04	0.21 ± 0.03	2.04 ± 0.17	0.65 ± 0.14	0.22 ± 0.14	0.24 ± 0.03	
	10C[a/,d]	38 ± 2[d]	0.49 ± 0.02	0.22 ± 0.01	2.05 ± 0.15	0.75 ± 0.07	0.13 ± 0.02	0.22 ± 0.03	
Female	0	36 ± 1[e]	0.46 ± 0.01	0.17 ± 0.01	1.77 ± 0.11	0.46 ± 0.02	0.24 ± 0.05		0.455 ± 0.323
	1	36 ± 3[c]	0.44 ± 0.03	0.22 ± 0.02[g]	1.57 ± 0.10	0.51 ± 0.10	0.14 ± 0.03		0.405 ± 0.127
	3	32 ± 1[b]	0.46 ± 0.01	0.15 ± 0.01	1.43 ± 0.08	0.52 ± 0.09	0.19 ± 0.03		0.451 ± 0.202
	10	34 ± 1[c]	0.44 ± 0.01	0.18 ± 0.01	1.69 ± 0.06	0.47 ± 0.04	0.13 ± 0.02		0.155 ± 0.098
	10C	36 ± 1[f]	0.47 ± 0.01	0.18 ± 0.01	1.80 ± 0.10	0.49 ± 0.05	0.21 ± 0.03		0.388 ± 0.191
			Relative Organ Weight (g/100 g body weight)						
Male	0		1.21 ± 0.05	0.56 ± 0.03	4.96 ± 0.36	1.79 ± 0.22	0.55 ± 0.18	0.54 ± 0.02	
	1		1.34 ± 0.10	0.69 ± 0.06	4.53 ± 0.13	1.80 ± 0.07	0.42 ± 0.07	0.54 ± 0.06	
	3		1.12 ± 0.09	0.52 ± 0.02	4.61 ± 0.39	1.75 ± 0.18	0.35 ± 0.07	0.56 ± 0.09	
	10		1.18 ± 0.05	0.54 ± 0.06	5.38 ± 0.78	1.69 ± 0.29	0.75 ± 0.42	0.64 ± 0.05	
	10C		1.29 ± 0.09	0.58 ± 0.01	5.41 ± 0.16	1.97 ± 0.10	0.36 ± 0.06	0.59 ± 0.06	
Female	0		1.31 ± 0.05	0.49 ± 0.02	5.04 ± 0.46	1.30 ± 0.05	0.65 ± 0.12		1.182 ± 0.807
	1		1.23 ± 0.12	0.62 ± 0.08[g]	4.42 ± 0.20	1.46 ± 0.20	0.39 ± 0.07		1.089 ± 0.239
	3		1.43 ± 0.03	0.46 ± 0.02	4.45 ± 0.27	1.59 ± 0.25	0.60 ± 0.09		1.333 ± 0.584
	10		1.29 ± 0.02	0.52 ± 0.03	4.93 ± 0.22	1.37 ± 0.09	0.39 ± 0.06		0.463 ± 0.300
	10C		1.31 ± 0.03	0.49 ± 0.02	4.98 ± 0.28	1.35 ± 0.09	0.58 ± 0.08		1.062 ± 0.508
			Relative Organ Weight (g/g brain weight)						
Male	0			0.47 ± 0.03	4.13 ± 0.32	1.50 ± 0.19	0.46 ± 0.15	0.45 ± 0.02	
	1			0.51 ± 0.03	3.44 ± 0.26	1.36 ± 0.08	0.32 ± 0.05	0.40 ± 0.03	
	3			0.47 ± 0.05	4.15 ± 0.25	1.58 ± 0.15	0.31 ± 0.05	0.49 ± 0.06	
	10			0.46 ± 0.03	4.57 ± 0.67	1.42 ± 0.21	0.67 ± 0.40	0.54 ± 0.03	
	10C			0.46 ± 0.03	4.26 ± 0.39	1.56 ± 0.18	0.27 ± 0.03	0.46 ± 0.03	
Female	0			0.38 ± 0.01	3.83 ± 0.24	1.00 ± 0.03	0.52 ± 0.12		0.906 ± 0.620
	1			0.51 ± 0.07[g]	3.67 ± 0.34	1.18 ± 0.08	0.32 ± 0.06		0.911 ± 0.233
	3			0.33 ± 0.02	3.11 ± 0.16	1.11 ± 0.18	0.42 ± 0.06		1.000 ± 0.456
	10			0.41 ± 0.03	3.82 ± 0.21	1.07 ± 0.09	0.30 ± 0.05		0.357 ± 0.230
	10C			0.37 ± 0.01	3.92 ± 0.21	1.03 ± 0.06	0.45 ± 0.06		0.842 ± 0.424

a/ Fed 10% cotton linters.
b/ Mean ± standard error of ten mice.
c/ Mean ± standard error of four mice.
d/ Mean ± standard error of three mice.
e/ Mean ± standard error of eleven mice.
f/ Mean ± standard error of twelve mice.
g/ Significantly different from control mice by Dunnett's multiple comparison procedure.

TABLE 21

SUMMARY OF LESIONS OF CONTROL MICE FED 24 MONTHS

Sex:	Male										Female										
Mouse Number:	227	233	234	236	238	240	241	248	252	256	344	347	348	349	350	351	352	354	355	356	357
Lesions[a/]																					
Adrenal Gland																					
Fibroplasia	1	1	1	1	1	1	1	1	1	2	2	1	1	2	1	1	2	1	2	2	2
Amyloid deposits	2	1	2		1		1	1	3	2				1	2		1	2		2	2
Thyroid Gland																					
Amyloid deposits	1																				
Cystic follicles											1		2		2		2				
Lung																					
Bronchoalveolar carcinoma		X	X				X		X										X		
Heart																					
Amyloid deposits	1	1	1	1	1	1	2		1	1					2			1	1	2	
Fibroplasia													1								
Blood Vessel																					
Hemangioma																					X
Liver																					
Amyloid deposits									1	1								1		1	
Nodular hyperplasia															1						
Cyst			X											X							
Hepatoma						X															
Lymphoma	X																				
Stomach																					
Amyloid deposits		1		2	2				1	1											
Epithelial hyperplasia												1		1							
Intestine																					
Amyloid deposits	3	3	2	3	3		3	3	3	3	1	1	1	1	3	3	2	3	3	3	3
Helminths	X	X		X			X	X	X	X											X
Lymphoma	X																				
Kidney																					
Amyloid deposits	3	3	3	3	3	1	3	3	3	3			3	2	3	1	3	3	3	3	3
Lymphoma	X																				
Testis																					
Mineralization of tubules							1														
Ovary																					
Amyloid deposits															3						
Cysts														X			X			X	
Uterus																					
Amyloid deposits															3						
Cystic endometrium											X	X	X	X				X			X
Hemangioma																X	X				
Eye																					
Retinal degeneration		1	1	1			1		1	1	1	1	1	1	1	1	1			1	1
Lens degeneration						1															
Skin																					
Ectoparasites												X				X	X				
Brain																					
Vacuoles in fiber tracts										1	1	1	2								
Spleen																					
Amyloid deposits		2		2																	
Lymphoma	X													X							
Hemangioma																X					
Lymph Node																					
Amyloid deposits															2						
Lymphoma	X													X							
Hemangioma												X									
Bone Marrow																					
Leukemia															X						
M/F ratio	1.5	1.4	1.1	4.0	1.5	2.0	1.0	1.7	1.0	1.2	1.9	1.3	1.1	1.0		1.3	1.6	1.5	1.	1.1	1.2

Tissues not listed were normal.

a/ Severity of lesions: 1 = slight; 2 = moderate; 3 = severe; X = present.

TABLE 72

SUMMARY OF LESIONS OF MICE FED 10% NC FOR 24 MONTHS

Sex:	Male				Female			
Mouse Number:	851	857	858	859	933	934	954	955
Lesions[a/]								
Adrenal Gland								
Fibroplasia	1	1	1	2		1	1	2
Amyloid deposits	2					2	1	1
Thyroid Gland								
Amyloid deposits					3		3	
Cystic follicles	2					2		1
Heart								
Amyloid deposits							2	
Fibroplasia				1				
Liver								
Necrosis						1		
Hepatoma			X					
Stomach								
Amyloid deposits	1						1	
Intestine								
Amyloid deposits	3		3		2	3	2	3
Helminths		X					X	
Kidney								
Amyloid deposits	3	3	1		1	3		3
End stage kidney							X	
Seminal Vesicle								
Hemorrhage			3					
Uterus								
Cystic endometrium					X	X	X	X
Eye								
Retinal degeneration						1	1	
Lens degeneration		1						
Skin								
Ectoparasites				X			X	X
Brain								
Vacuoles in fiber tracts							1	1
Skeletal Muscle								
Sarcosporidia						X		
Spleen								
Amyloid deposits					1		2	
Lymph Node								
Hemangioma					X			X
Bone Marrow								
M/E ratio	0.9	1.3	-	1.0	-	1.2	1.5	1.6

Tissues not listed were normal.

a/ Severity of lesions: 1 = slight; 2 = moderate; 3 = severe; X = present.

TABLE 73

SUMMARY OF LESIONS OF CONTROL MICE DYING AT UNSCHEDULED TIMES

Sex:	Male						Female					
Mouse Number:	214	251	245	247	221	250	317	324	332	325	326	314
Week of Death	79	89	94	94	97	104	44	60	81	103	103	108[b]
Lesions[a]												
Adrenal Gland												
Fibroplasia		1		2	1				1	1		
Amyloid deposits	3		1	1	3						1	3
Thyroid Gland												
Amyloid deposits					3						2	3
Cystic follicles			1			1						
Heart												
Amyloid deposits	1		1	1							1	
Lung												
Pneumonia						1						
Pleuritis		X										
Bronchoalveolar carcinoma		X		X					X			
Salivary Gland												
Fibroplasia						1						
Liver												
Amyloid deposits					1							
Necrosis											1	
Stomach												
Amyloid deposits	1			1	2						3	2
Pancreas												
Amyloid deposits											3	2
Intestine												
Amyloid deposits	3		1		3	3	2		2	1	3	3
Helminths		X	X	X	X	X	X		X		X	X
Kidney												
Amyloid deposits	3	1	1	2	3	3				1	3	3
Testis												
Epithelial degeneration						1						
Epididymis												
Cyst						1						
Ovary												
Amyloid deposits										3		3
Cysts											X	
Uterus												
Cystic endometrium									X		X	X
Hemangioma										X		
Eye												
Retinal degeneration	1		1	1	1						1	1
Skin												
Ulceration, scarring			X				X					
Brain												
Vacuoles in fiber tracts								2	1	1	2	3
Mammary Gland												
Adenocarcinoma									X			
Spleen												
Amyloid deposits					3	1						3
Lymph Node												
Amyloid deposits			2									
Bone Marrow												
M/E ratio	1.0	-	2.0	2.1	1.3	-	-	-	1.0	1.0	1.0	-

Tissues not listed were normal.

a/ Severity of lesions: 1 = slight; 2 = moderate; 3 = severe; X = present.

b/ Died in week 4 of recovery after 24 months feeding.

TABLE 74

SUMMARY OF LESIONS OF MALE MICE FED 10% NC AND DYING AT UNSCHEDULED TIMES

Mouse Number:	831	847	843	819	808	816	820	813	831	844	828	812	818	835	823	830
Week of Death:	8	10	16	33	35	35	35	41	45	45	64	81	85	96	97	97
Lesions[a/]																
General Autolysis	X			X						X						
Adrenal Gland																
Fibroplasia					1							2	2		2	
Amyloid deposits													1	2	2	3
Pituitary																
Cysts																1
Heart																
Amyloid deposits															2	3
Fibroplasia	1													1		
Mural thrombus														X	X	
Lung																
Pneumonia												1				
Liver																
Amyloid deposits															2	1
Centrolobular change, acute											2					
Salivary Gland																
Amyloid deposits														3	2	3
Pancreas																
Amyloid deposits															1	
Stomach																
Amyloid deposits														2	2	
Intestine																
Amyloid deposits													1	2	3	3
Helminths			X								X	X				
Nematodes														X	X	
Kidney																
Amyloid deposits														3	3	3
Eye																
Adenoma												X				
Skin																
Ectoparasites																X
Brain																
Vacuoles in fiber tracts			2				1	1			1					
Spleen																
Amyloid deposits														2	3	
Lymphocyte necrosis	1	1						1			1					
Bone Marrow																
M/E ratio	-	-	-	-	-	-	-	-	-	-	-	-	2.0	1.0	2.0	2.0

Tissues not listed were normal.

a/ Severity of lesions: 1 = slight; 2 = moderate; 3 = severe; X = present.

TABLE 75

SUMMARY OF LESIONS OF FEMALE MICE FED 10% NC AND DYING AT UNSCHEDULED TIMES

Mouse Number:	952	904	908	949	946	921	922	924	951	926
Week of Death:	5	8	21	23	82	85	85	85	89	102
Lesions[a]										
Adrenal Gland										
Fibroplasia				1					2	1
Amyloid deposits										2
Heart										
Amyloid deposits										1
Liver										
Centrolobular necrosis			2			2				
Intestine										
Amyloid deposits										2
Helminths		X								X
Ulcer				1						
Kidney										
End stage kidney					X					
Ovary										
Cystic						X				
Uterus										
Cystic endometrium						X	X	X	X	X
Eye										
Retinal degeneration				1						1
Skin										
Ectoparasites							X			X
Ulceration, scarring									X	
Brain										
Vacuolation of fiber tracts			2	1	1		1			2
Spleen										
Lymphocyte necrosis	X			X						
Bone Marrow										
M/E ratio	-	-	-	-	-	-	1.2	-	1.3	1.0

Tissues not listed were normal.

a/ Severity of lesions: 1 = slight; 2 = moderate; 3 = severe; X = present.

TABLE 76

INCIDENCE OF TUMORS IN MICE FED NC

Dose (% in Feed):	0		10	
Sex:	Male	Female	Male	Female
Site, Tumor				
Lung				
Bronchiolalveolar carcinoma	6[a/]	2		
Liver				
Hepatoma	3		1	
Uterus				
Hemangioma		2		
Eye				
Adenoma			1	
Lymph node				
Hemangioma				2
Mammary gland				
Adenocarcinoma		1		
Bone marrow				
Leukemia		1		
Unidentified site				
Hemangioma				1
Multiple sites				
Lymphoma	1	1		
Hemangioma		1		

a/ Number of mice with the lesion.

TABLE 77

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MICE FED NC FOR 24 MONTHS AND ALLOWED TO RECOVER FOR 1 MONTH

Sex	Dose (% in feed)	Terminal Body Weight (g)	Brain	Heart	Liver	Kidney	Spleen	Testis	Ovary
			Absolute Organ Weight (g)						
Male	0	37 ± 3[b/]	0.45 ± 0.02	0.22 ± 0.03	1.46 ± 0.04	0.65 ± 0.08	0.11 ± 0.03	0.21 ± 0.03	
	1	42 ± 1[b/]	0.47 ± 0.01	0.25 ± 0.01	1.98 ± 0.21	0.88 ± 0.07	0.18 ± 0.04	0.23 ± 0.01	
	3	40 ± 2[c/]	0.47 ± 0.02	0.32 ± 0.04	1.63 ± 0.10	0.63 ± 0.05	0.10 ± 0.02	0.20 ± 0.02	
	10	36 ± 1[d/]	0.44 ± 0.03	0.24 ± 0.02	1.41 ± 0.06	0.55 ± 0.03	0.09 ± 0.03	0.16 ± 0.02	
Female	0	33 ± 3[b/]	0.48 ± 0.01	0.17 ± 0.02	1.64 ± 0.27	0.46 ± 0.03	0.23 ± 0.04		0.220 ± 0.119
	1	37 ± 3[b/]	0.49 ± 0.02	0.18 ± 0.01	1.44 ± 0.15	0.51 ± 0.05	0.19 ± 0.04		0.457 ± 0.412
	3	29 ± 2[c/]	0.47 ± 0.01	0.17 ± 0.01	1.23 ± 0.03	0.42 ± 0.04	0.13 ± 0.03		0.078 ± 0.034
	10	38 [e/]	0.49	0.20	1.67	0.60	0.13		0.140
	10C[a/]	34 ± 2[c/]	0.46 ± 0.02	0.18 ± 0.01	1.39 ± 0.07	0.50 ± 0.04	0.13 ± 0.02		0.105 ± 0.037
			Relative Organ Weight (g/100 g body weight)						
Male	0		1.22 ± 0.03	0.60 ± 0.06	3.95 ± 0.20	1.74 ± 0.15	0.29 ± 0.07	0.56 ± 0.09	
	1		1.11 ± 0.01	0.59 ± 0.04	4.70 ± 0.43	2.08 ± 0.12	0.42 ± 0.09	0.56 ± 0.03	
	3		1.21 ± 0.10	0.81 ± 0.09	4.18 ± 0.39	1.61 ± 0.23	0.26 ± 0.07	0.52 ± 0.07	
	10		1.22 ± 0.05	0.67 ± 0.04	3.90 ± 0.07	1.54 ± 0.05	0.27 ± 0.08	0.45 ± 0.07	
Female	0		1.47 ± 0.10	0.53 ± 0.03	4.95 ± 0.57	1.40 ± 0.03	0.68 ± 0.10		0.734 ± 0.456
	1		1.32 ± 0.08	0.48 ± 0.04	3.84 ± 0.28	1.36 ± 0.14	0.51 ± 0.11		1.297 ± 1.186
	3		1.66 ± 0.14	0.59 ± 0.03	4.42 ± 0.41	1.53 ± 0.26	0.47 ± 0.10		0.258 ± 0.097
	10		1.29	0.53	4.40	1.58	0.34		0.368
			Relative Organ Weight (g/g brain weight)						
Male	0			0.49 ± 0.05	3.24 ± 0.11	1.43 ± 0.13	0.24 ± 0.06	0.46 ± 0.06	
	1			0.53 ± 0.04	4.24 ± 0.42[f/]	1.88 ± 0.13	0.38 ± 0.08	0.50 ± 0.03	
	3			0.67 ± 0.06	3.45 ± 0.08	1.33 ± 0.12	0.21 ± 0.04	0.43 ± 0.05	
	10			0.54 ± 0.01	3.20 ± 0.17	1.26 ± 0.01	0.22 ± 0.07	0.37 ± 0.07	
Female	0			0.36 ± 0.03	3.41 ± 0.52	0.96 ± 0.06	0.47 ± 0.09		0.464 ± 0.256
	1			0.37 ± 0.03	2.96 ± 0.40	1.04 ± 0.13	0.39 ± 0.09		0.899 ± 0.806
	3			0.36 ± 0.02	2.65 ± 0.07	0.90 ± 0.08	0.29 ± 0.07		0.167 ± 0.075
	10			0.41	3.41	1.22	0.27		0.286
	10C			0.41 ± 0.04	3.04 ± 0.16	1.09 ± 0.07	0.28 ± 0.03		0.223 ± 0.074

a/ Fed 10% cotton linters.
b/ Mean ± standard error of three mice.
c/ Mean ± standard error of four mice.
d/ Mean ± standard error of two mice.
e/ One surviving mouse.
f/ Significantly different from control mice by Dunnett's multiple comparison procedure.

VI. GENERAL DISCUSSION AND CONCLUSIONS

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VI. GENERAL DISCUSSION AND CONCLUSIONS

A. Toxic Effects

The main effects seen in these studies were "fiber effects" in animals fed either 10% nitrocellulose or 10% cotton linters. All three species, dogs, rats, and mice, had a dose-related increase in total feed consumption. This is what one would expect if the fibers were merely inert bulk passing through the gastrointestinal tract with no absorption at all, as shown in our earlier study with ^{14}C-NC in rats.[6] In view of the similarities in results between rodents and carnivores, it is likely that there is no absorption of oral doses of NC in any species of mammals except for cattle and other ruminants capable of digesting cellulose.

Since the dogs usually ate their feed or left it in the bowl with little scattering, it was easy to determine the amount consumed. With rodents, this was more difficult, since they scattered some of their feed. The scattering of feed was negligible at the 1% and 3% dietary levels, but very obvious in both 10% fiber mixtures. In large part, the scattering appeared to be an attempt by the rodents to separate the fiber from the nutrients. Because of this effect, the measured feed-consumption (in part, feed removal by the rodent) data for the high dose and cotton control rodents were higher than the actual amount consumed. Attempts to quantitate the amount of scattered fiber and feed were not completely successful, especially with the linters, because of the impossibility of reducing the bales to individual fibers to form a completely homogeneous blend.

With rodents, there was a decrease of weight gain in the high dose and cotton control groups. In the first week of feeding, there were weight decreases due to adaptation problems. However, this lower body weight is not necessarily an adverse effect. When rodents are allowed to feed ad libitum, they tend to overeat and become grossly obese. The rodents fed 10% fiber were characterized by less fat, not less lean body mass. Nevertheless, a decreased nutrient intake can be deterimental if there is a very high body demand for nutrition. This condition apparently occurred, to some degree, in the earlier litters of the three-generation study in rats. The 10% cotton females of the F_0 and F_1 generations had decreased pup survival during lactation and decreased pup weights. The lack of such an effect in the F_2 females may have been due to adaptation.

The only species with obvious toxic effects was the mouse. These occurred in 10% fiber groups only. First, deaths occurred in the first 3 weeks of feeding from intestinal impaction. As noted earlier,[6] the fibers of both NC and linters were large enough, relative to the lumen of the intestine in mice, that the masses formed by the fibers could completely

obstruct the gut. Interestingly enough, this type of death was not seen after the third week of feeding. The second adverse effect was the "red ear syndrome," or hyperemia with edema. It was not a life-threatening condition, and resolved spontaneously. The cause of this is unknown. Irritation from fiber contact seems to be the possible likely explanation. Finally, there was a spate of deaths about Month 9. The deaths in the high dose mice were three times the deaths in the cotton control mice. This effect could have been due, in part, to the chemical difference between the fibers. The total deaths over the 2-year test period did not increase much; rather, the more susceptible mice died earlier than in the lower dosage groups. The cause is unknown. A possible explanation is an interaction between fiber ingestion or contact, and unknown environmental factors.

B. Conclusions

Nitrocellulose acts as non-nutritive bulk with no adverse effects at levels up to 10% of the diet in dogs and rats and 3% of the diet in mice.

This dietary bulk gave some adverse effects in rats with an extremely high nutrient requirement, such as during pregnancy and lactation.

NC, at 10% in the diet, could kill mice by intestinal impaction: this is due to its fibrous nature. A spate of deaths of unknown cause occurred after 9 months of feeding 10% NC: this may have been due, in prt, to NC's chemical composition (i.e., nitro groups). Therefore, it is not certain whether 10% NC is more toxic or as toxic to mice as 10% cotton linters.

C. Water Quality Criterion

1. Rationale

A water quality criterion for a compound deals with the risk to man, and is related not only to the population in general but to special groups at risk. Existing standards for the populations occupationally exposed, although based on toxicologic data as is the water quality criterion, has been calculated specifically for occupational exposure rather than environmental exposure. Special groups at risk in the environmental case would include not only populations exposed to the chemicals in air and water, but in consumer products in which the chemicals might be found. With the compounds above, special groups at risk would be: workers involved in their synthesis; persons involved in production/use of propellants; and persons using derivatives as plastics. Since data to quantitate these special exposures were not gathered as part of the water criteria,

the information used was from laboratory animal toxicity tests, calculated using the guidelines and methods as outlined by the EPA.[17]

2. Results and Conclusions

Although these studies were of good quality, with very high doses in three species, no toxic effects of NC, per se, were found. In view of its low solubility and apparent non-absorption,[6] this is not surprising.

Therefore, we conclude that the only appropriate water quality criteria are physical ones, such as clarity and total suspended solids.

REFERENCES

1. Lee, C. C., J. V. Dilley, J. R. Hodgson, D. N. Roberts, D. O. Helton and W. J. Wiegand. Mammalian Toxicity of Munition Compounds: Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization and Disposition and Metabolism. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 1:1-100, 22 July 1975. NTIS Report No. AD-B011,150.

2. Lee, C. C. et al. Mammalian Toxicity of Munition Compounds: Phase I. Supplement. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, Disposition and Metabolism and Ames Test. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 6:1-39 (1978).

3. Lee, C. C., H. V. Ellis III, J. J. Kowalski, J. R. Hodgson, S. W. Hwang, R. D. Short, J. C. Bhandari, J. L. Sanyer, T. W. Reddig, J. L. Minor and D. O. Helton. Mammalian Toxicity of Munition Compounds: Phase II. Effects of Multiple Doses. Part I. Trinitroglycerin. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 2:1-73 (1977). NTIS Report No. AD-A047,067.

4. Lee, C. C., H. V. Ellis III, J. J. Kowalski, J. R. Hodgson, S. W. Hwang, R. D. Short, J. C. Bhandari, J. L. Sanyer, T. W. Reddig and J. L. Minor. Mammalian Toxicity of Munition Compounds: Phase II. Effects of Multiple Doses. Part II. 2,4-Dinitrotoluene. USAMRDC Contract No. DAMD-74-17-C-4073. Report No. 3:1-171 (1977). NTIS Report No. AD-A061,715.

5. Lee, C. C., H. V. Ellis III, J. J. Kowalski, J. R. Hodgson, R. D. Short, J. C. Bhandari, T. W. Reddig and J. L. Minor. Mammalian Toxicity of Munition Compounds: Phase II. Effects of Multiple Doses. Part III. 2,6-Dinitrotoluene. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 4:1-144 (1978). NTIS Report No. AD-A062,015.

6. Ellis, H. V. III, J. J. Kowalski, J. R. Hodgson, J. C. Bhandari, J. L. Sanyer, T. W. Reddig, J. L. Minor and C. C. Lee. Mammalian Toxicity of Munition Compounds: Phase II. Effects of Multiple Doses. Part IV. Nitrocellulose. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 5:1-94 (1978). NTIS Report No. AD-A062,016.

7. Ellis, H. V. III, J. H. Hagensen, J. R. Hodgson, J. L. Minor, C. B. Hong, E. R. Ellis, J. D. Girvin, D. O. Helton, and B. L. Herndon. **Mammalian Toxicity** of Munitions Compounds: Phase III. Effects of Life-time Exposure. Part I. 2,4Dinitrotoluene. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 7:1-253 (1978).

8. Ellis, H. V. III, J. H. Hagensen, J. R. Hodgson, J. L. Minor, C. B. Hong, E. R. Ellis, J. D. Girvin, D. O. Helton, and B. L. Herndon. Mammalian Toxicity of Munitions Compounds: Phase III. Effects of Life-time Exposure. Part II. Trinitroglycerin. USAMRDC Contract No. DAMD-17-74-C-4073. Report No. 8:1-193 (1978).

9. Reisman, R. E. and C. E. Arbesman. Systemic Allergic Reactions Due to Inhalation of Penicillin. J. Am. Med. Assoc., 203:986 (1968).

10. Mancini, G., A. O. Carbonara and J. F. Heremans. Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion. Immunochemistry, 2:235 (1964).

11. Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips and D. A. Hungerford: Chromosome Preparations of Leukocytes Cultured from Human Peripheral Blood. Expt. Cell Res., 20:613 (1960).

12. Eggen, R. R. Cytogenetics. In Clinical Diagnosis by Laboratory Methods, Davidsohn and Henry (eds.), W. B. Saunders, Philadelphia, pp. 1209-1240 (1969).

13. Fernandes, M. V. The Development of a Human Amnion Strain of Cells. Texas Repts. Biol. Med., 16:48 (1958).

14. Vogt, M., and R. Dulbecco. Virus-Cell Interaction With Tumor Producing Virus. Proc. Nat. Acad. Sci., 46:365 (1960).

15. Moorhead, P. S. and P. C. Nowell. Chromosome Cytology. In Methods in Medical Research (H. N. Eisen, Editor). Yearbook Medical Publ. Inc., Chicago, 10:310 (1964).

16. MacKenzie, William F. and F. M. Garner. Comparison of Neoplasms in Six Sources of Rats. J. Nat. Cancer Inst., 50:1243 (1973).

17. Environmental Protection Agency. Water Quality Criteria. Fed. Reg. 44:15926-15981 (1979).

18. Helton, Danny O. Chemical and Physical Characterization of Nitrocellulose Fines. USAMRDC Contract No. DAMD-17-74-C-4073. Special Report: 1-23 (1976).

19. Selig, Walter. Microdetermination of Aromatic Nitro Compounds, Nitrocellulose, and Cyclic Nitramines. AEC Report UCRL-6639:20-28 (1961).

APPENDIX I

MANUAL FOR

HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

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HEMATOLOGY, CLINICAL LABORATORY TESTS, HISTOPATHOLOGY, STATISTICAL ANALYSIS, AND NORMAL VALUES

I. HEMATOLOGY AND CLINICAL LABORATORY TESTS

The usual blood sample from dogs is 8 ml, from monkeys 4 ml, and from rats 0.3 ml for hematology and about 8 ml for full analysis at termination.

A. Hematology

The following hematological analyses are performed on all blood samples from rats, dogs and monkeys.

1. Erythrocyte and leukocyte counts: A Coulter Electronic Particle Counter with 100 μ aperture is used.[1] Particle-free diluents (Isoton for RBC, Zap-Oglobin in Isoton for WBC, Coulter Electronics, Inc.) are counted to establish the background. Each blood sample is counted in duplicate. For each test day, two control blood samples (Diagnostic Technology, Inc.) are counted separately in duplicate.

2. Hematocrit: Hematocrit is determined in capillary tubes using a microcapillary centrifuge (International Equipment Company, Model MB). Two control blood samples (Diagnostic Technology, Inc.) are measured separately in duplicate.

3. Hemoglobin: Hemoglobin is measured as cyanomethemoglobin.[2] Each blood sample is measured in duplicate. Cyanomethemoglobin (Coulter Electronics, Inc.) is used as the standard. For each assay, two levels of the standard are used and two control blood samples (Diagnostic Technology, Inc.) are measured in duplicate.

4. Methemoglobin (Met-Hb): Met-Hb is measured by the method of Dubowski.[3] A positive control is made by adding potassium ferricyanide to control blood.

5. Heinz bodies: Heinz bodies are stained with methyl violet and the percent of Heinz bodies is calculated.

6. Mean corpuscular volume (MCV): MCV is calculated as follows:

$$\text{MCV } (\mu^3) = \frac{\text{Hematocrit} \times 10}{\text{Erythrocytes in millions/mm}^3}$$

7. Mean corpuscular hemoglobin (MCHb): MCHb is calculated as follows:

$$\text{MCHb } (\mu\mu g) = \frac{\text{Hemoglobin (gm \%) x 10}}{\text{Erythrocytes in millions/mm}^3}$$

8. Mean corpuscular hemoglobin concentration (MCHbC): MCHbC is calculated as follows:

$$\text{MCHbC (gm \%)} = \frac{\text{Hemoglobin (gm \%) x 100}}{\text{Hematocrit}}$$

9. Differential leukocyte counts: Wright's stain is used to stain the leukocytes for examination.

10. Reticulocyte count: Reticulocytes are counted by the methylene blue method using the Miller disc.[4]

11. Platelet count: A Coulter Electronic Particle Counter with 70 μ aperture is used.[5] Particle-free Isoton is used as diluent and counted to establish the background. At weekly intervals, platelets are also visually counted in a hemocytometer with a phase microscope for comparison.[6]

12. Clotting time (dog and monkey): Clotting time is determined by the capillary tube procedure using two capillary tubes.[7] The time elapsed from the appearance of the blood from the animal and coagulation in either tube is measured.

B. Clinical Blood Tests

The following clinical blood chemistry tests are performed on all blood samples from dogs and monkeys and on blood samples from rats at termination.

1. Blood glucose: Fasting blood glucose is determined by Stein's hexokinase method.[8] Standard glucose solution (Dade) is used to establish a standard curve. For each assay, one level of the standard and two controls (Reference Serum, Worthington; and Validate, General Diagnostics) are measured.

2. Serum glutamic-oxaloacetic transaminase (SGOT): SGOT is measured by the method of Amador and Wacker.[9] Validate and Reference Serum are used as the enzyme reference for each assay.

3. Serum glutamic-pyruvic transaminase (SGPT): SGPT is measured by the method of Henry et al.[10] Validate and Reference Serum are used as the enzyme reference for each assay.

4. Alkaline phosphatase: Alkaline phosphatase is measured by the method of Bowers and McComb.[11] Validate and Reference Serum are used as the enzyme reference for each assay.

5. BUN: BUN is measured using the BUN Strate Kit (General Diagnostic) which is based on the urease method.[12] Three levels of Calibrate (General Diagnostics) are used to establish a standard curve. For each assay, two controls (Calibrate I and Validate) are used as the reference.

6. Creatinine: Creatinine is measured by a modified kinetic alkaline picrate procedure.[13] Creatinine Standard Solutions (Sigma Chemical Company) are used to establish a standard curve. For each assay, two levels of the standard and two controls (Calibrate I and Validate) are used as reference.

7. Lactate dehydrogenase (LDH): LDH is measured by the method of Wacker et al.[14] Precinorm E and Precipath E (Boehringer, Mannheim Corporation) are used as the enzyme controls for each assay.

8. α-Hydroxybutyrate dehydrogenase (α-HBDH): α HBDH is measured by the method of Rosalki and Wilkinson.[15] Precinorm E and Precipath E are used as the enzyme controls for each assay.

9. Creatine phosphokinase (CPK): CPK is measured by the improved procedure of Rosalki[16] based on the methods of Oliver.[17] Precinorm E and Precipath E are used as the enzyme controls for each assay.

C. Urinalysis

Urine samples are collected from animals before and during treatment as are the blood samples. The urine from rats is collected by slight manipulation of their body, and samples within each group are pooled. The monkeys and dogs are placed individually in metabolism cages, and urine is collected in the stainless steel pan. The urine from each dog and the pooled urine from rats are tested and examined for the following:

1. Protein: Urinary protein is determined with Labstix (Ames Company, Elkhart, Indiana).

2. Sugar: Urinary glucose and reducing substance are determined with Labstix (Ames Company).

3. Microscopic examination: Urine samples are centrifuged and the supernatant discarded. The residue is resuspended and examined microscopically for the presence of erythrocytes, leukocytes, epithelial cells, and crystals under high power field and for casts under low power field.

A positive urine control prepared with known amounts of protein and glucose in saline adjusted to pH 6.0 is run with each assay to check the reliability of the Labstix.

D. Occult Blood in Feces

Fecal samples are collected from animals before and during treatment as are the blood and urine samples. Occult blood in the feces is determined with Hematest Reagent Tablets (Ames Company, Elkhart, Indiana). A positive control (whole blood) and a negative control (distilled water) are included with each assay to check the reliability of the Hematest tablets.

E. Precision of Hematology and Clinical Blood Chemistry Tests

1. Reproducibility

For erythrocyte and leukocyte counts, hematocrit, hemoglobin, and the various clinical blood chemistry tests, the same control blood samples or control standards are used for day-to-day assays. The replication of results are excellent and are summarized in Table A.

The determination of differential leukocyte counts and reticulocyte counts are performed by experienced personnel. At weekly intervals, a blood sample is counted by two or more personnel to confirm the accuracy of the counting. Also at weekly intervals, the platelet counts obtained from a Coulter Electronic Particle Counter are compared with the direct visual counts in a hemocytometer using a phase microscope.

2. Reproducibility Within a Test Day

At monthly intervals, a blood sample is taken from a control dog and six or more determinations for erythrocyte, leukocyte, reticulocyte, and platelet counts, hemoglobin, and various clinical blood chemistry tests are performed to establish the reproducibility within an assay. The results are summarized in Table B.

3. Proficiency Test Service

We subscribe to the Proficiency Test Service of the Institute for Clinical Science, Hahnemann Medical College, Philadelphia, Pennsylvania (F. Wm. Sunderman, M.D., Director). On the first day of each month, this service sends two samples containing two different sera or solutions to all subscribers for measurements of one or more of the parameters usually analyzed in clinical laboratories. Participants report their results on a form furnished by the service. On the 15th day of the month, each participant receives a report from the service which includes: the results of a statistical analysis of the values reported by all the participating laboratories; a current review of pertinent methodology; a comprehensive bibliography; and validation of the results which the participating laboratory reported. This service enables each participating laboratory to obtain an unbiased and critical assessment of its proficiency in relation to that of 1,000 or so other clinical laboratories throughout the country. The service has been in continuous operation since 1949 and was given endorsement by the American Society of Clinical Pathologists in 1952 and by the Association of Clinical Scientists in 1957 and 1968. Our results have been found to be satisfactory and are summarized in Table C.

II. HISTOPATHOLOGY

A. Necropsy and Gross Examination

At termination or prior to imminent death, rats are killed with ether, and dogs and monkeys with an overdose of sodium pentobarbital. Animals that die on tests are kept refrigerated but not frozen until necropsy. The general physical condition and nutritional status of each animal at the time of death or termination are observed and recorded. Necropsy is performed as soon as possible after death. Gross changes of all tissues are carefully examined and recorded.

B. Organ Weights

The brain, liver, spleen, kidneys, adrenals, thyroids and gonads are trimmed free from surrounding tissues and weighed. The organ weight to body weight and/or brain weight ratios are then calculated.

C. Tissues for Microscopic Examination

Tissues to be examined include the eye, skin (breast), trachea, lung, tongue (except rat), salivary gland, liver, gallbladder (except rats), pancreas, esophagus, fundic and pyloric stomach, duodenum, jejunum, ileum, cecum, colon, kidneys, urinary bladder, gonads, and accessory organs, diaphragm and gracilis muscle, anterior pituitary, thyroids/parathyroids, adrenals, tonsil (except rat), thymus, spleen, prescapular (except rats) and mesenteric lymph nodes, rib bone with bone marrow, brain (sagittal section for rats; coronal sections of cerebral cortex, cerebellum, and brain stem for dog and monkey), spinal cord (lumbosacral plexus, dog and monkey), sciatic nerve and any other structures not mentioned which show abnormal gross changes.

D. Fixation and Staining of Tissues

All tissues are cut not to exceed 1 cm in thickness for fixation. For most tissues, neutral buffered 10% formalin is used. Sufficient volume of fixing solution is used and the tissues are changed to a fresh solution after 24 hours. The fixed tissues are processed in an Autotechnicon for dehydration, clearing, and infiltration and then embedded in paraffin. Routine H & E staining is used to stain the sectioned tissues for microscopic examination.

Supplementary tissue fixatives and staining techniques may be employed for more positive identification of special lesions such as calcification, pigments, fat deposition and other abnormal changes.

III. STATISTICAL ANALYSIS

Data are analyzed statistically using the Dunnett's multiple comparison procedure following an analysis of variance,[18] or our modification of this procedure for uneven numbers among groups. The chosen criterion significance is $p < 0.05$. The means of each group at various intervals during treatment are compared with pretreatment levels. For most experiments in beagles, three baseline (pretreatment) levels are obtained. The baseline levels for each animal are averaged and the mean is used in the analysis. In addition, the means of the various treated groups are compared with that of the control group at the respective time intervals.

IV. NORMAL VALUES

A. Hematology, Clinical Laboratory Tests and Bone Marrow

Since June 1971, we have used about 180 rhesus monkeys (Woodard Research Corporation, Herndon, Virginia, Primate Imports, Port Washington, New York, and PrimeLabs, Inc., Farmingdale, New Jersey) for various studies. The peripheral blood elements and clinical blood chemistry values of these monkeys before treatment and the myeloid/erythroid (M/E) ratio of the bone marrow of the monkeys used as normal controls varied among individual animals. The mean ± S.D. and the range of the various parameters for the males and females are summarized in Tables D and E, respectively.

Since September 1971, we have used about 525, 5 to 9 months old, beagles dogs (AKC registered, Hazelton Research Animals, Inc.). The peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow varied considerably among individual dogs. The mean ± S.D. and the ranges of the various parameters for the males and females are summarized in Tables H and I, respectively.

During the same period, we have used about 500, 7 to 10 weeks old, male albino rats (CD® Strain, Charles River Breeding Laboratories). As for the dogs, the individual variations of the peripheral blood elements, clinical blood chemistry values and the M/E ratio of the bone marrow were large. The mean ± S.D. and the ranges of the various parameters for these male rats are summarized in Table L.

B. Absolute and Relative Organ Weights

Organ weights, both absolute and relative to body weight, of rhesus monkeys, beagle dogs, and albino rats are summarized in Tables F and G, J and K, and M, respectively. These were control animals used between June 1971 and December 1976.

C. Presence of Various Substances in the Urine

Various substances occasionally occurred in the urine of monkeys, dogs and rats. The results are summarized in Table N. Large percentage of urine samples from monkeys contained epithelial cells, i.e., 34.7% to 52.0%. Other substances occurred in 8.1% or less of the urine samples.

In dogs, protein, erythrocytes, leukocytes and epithelial cells were present in 19.1 to 21.6%, 16.5 to 19.8%, 22.6 to 24.6% or 24.7 to 25.7%, respectively, of the samples from dogs collected for analysis. Glucose,

crystals, and casts occurred in less than 2% of these samples. Some dogs had been bled and returned to the metabolism cages before the urine was removed for analysis. The high incidence of some of these substances in the urine of these dogs might be due to contamination with the fecal material and traces of blood dropped in the cage. Special care to avoid contamination has been undertaken.

In rats, large percentage of urine samples contained protein, i.e., 29.8 to 36.0%. A few samples contained erythrocytes, leukocytes, epithelial cells and crystals.

D. Occult Blood in the Feces

Less than 10% of the feces samples from monkeys or dogs was positive with the Hematest for occult blood. The results are summarized in Table O.

V. REFERENCES

1. Brecher, G., M. Schneiderman, and C. Z. William: Evaluation of the Electronic Red Cell Counter. Am. J. Clin. Path., 26: 1439, 1956.

2. Selegson, D.: Standard Methods of Clinical Chemistry, Academic Press, Inc., New York, Vol. 2, p. 52, 1958.

3. Dubowski, K. M.: Measurement of Hemoglobin Derivatives in Hemoglobin, Its Precusors and Metabolites. (F. W. Sunderman and F. W. Sunderman, Jrs., eds.), J. B. Lippincott Company, Philadelphia, p. 29, 1964.

4. Brecher, G., and M. Schneiderman: A Time-Saving Device for the Counting of Reticulocytes. Am. J. Clin. Path., 20: 1079, 1950.

5. Bull, B. S., M. A. Schneiderman, and G. Brecher: Platelet Counts With the Coulter Counter. Am. J. Clin. Path., 44: 678-688, 1965.

6. Brecher, G., M. Schneiderman, and E. P. Cronkite: The Reproducibility and Constancy of the Platelet Count. Am. J. Clin. Path., 23: 15, 1953.

7. Hepler, O. E.: Manual of Clinical Laboratory Methods, p. 83, Charles C. Thomas, Springfield, Illinois, 1935.

8. Slein, M. W.: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), p. 117, Academic Press, New York, 1963.

9. Amador, E., and W. E. C. Wacker: Serum Glutamic-Oxaloacetic Transaminase Activity: A New Modification and an Analytical Assessment of Current Assay Technics. Clin. Chem., 8: 343, 1962.

10. Henry, R. J., N. Chiamori, O. J. Golub, and S. Berkman: Revised Spectrophotometric Methods for the Determination of Glutamic-Oxaloacetic Transaminase, Glutamic-Pyruvic Transaminase, and Lactic Dehydrogenase. Am. J. Clin. Path., 34: 381, 1960.

11. Bowers, G. N., Jr., and R. B. McComb: A Continuous Spectrophotometric Method for Measuring the Activity of Serum Alkaline Phosphatase. Clin. Chem., 12: 70, 1966.

12. Chaney, A. L., and E. P. Manback: Modified Reagents for Determination of Urea and Ammonia. Clin. Chem., 8: 130, 1962.

13. Lustgarten, J. A.: A Simple, Rapid, Kinetic Method for Creatinine Concentration. Clin. Chem., 18: 1419, 1972.

14. Wacker, W. E. C., D. D. Ulmer, and B. L. Vallee: Metalloenzymes and Myocardial Infarction. II. Malic and Lactic Dehydrogenase Activities and Zinc Concentrations in Serum. New Eng. J. Med., 225, 449, 1956.

15. Rosalki, S. B., and J. H. Wilkinson: Reduction of α-Ketobutyrate by Human Serum. Nature (London), 188, 1110, 1960.

16. Rosalki, J. B.: An Improved Procedure for Serum Creatine Phosphokinase Determination. J. Lab. Clin. Med., 69, 696, 1967.

17. Oliver, I. T.: A Spectrophotometric Method for the Determination of Creatine Phosphokinase and Myokinase. Biochem. J., 61, 116, 1955.

18. Dunnett, C. W.: A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J. Am. Stat. Assoc., 50: 1096-1121, 1955.

TABLE A

REPRODUCIBILITY AMONG TEST DAYS ON THE SAME CONTROL SAMPLES OR STANDARDS[a/]

	No. of Determinations	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)			
Normal level	20	4.51 ± 0.07	4.36 - 4.67
Abnormal level	20	2.32 ± 0.04	2.25 - 2.40
Hematocrit (vol %)			
Normal level	20	44.3 ± 0.40	44 - 45
Abnormal level	20	22.8 ± 0.60	22 - 24
Hemoglobin (gm %)			
Normal level	20	14.2 ± 0.20	13.6 - 14.5
Abnormal level	20	7.4 ± 0.20	6.9 - 7.8
Leukocyte Counts (x 10^3/mm^3)			
Normal level	20	7.3 ± 0.50	6.8 - 8.7
Abnormal level	20	17.6 ± 0.80	16.3 - 18.7
Fasting Blood Glucose (mg %)	20	163.0 ± 7.5	151 - 178
SGOT (IU/ℓ)	23	61.7 ± 3.9	55 - 68
SGPT (IU/ℓ)	23	51.3 ± 2.6	46 - 55
Creatinine (mg %)	18	2.2 ± 0.3	1.6 - 2.6
BUN (mg %)	19	9.3 ± 0.2	9.5 - 10.2
Bilirubin (mg %)	11	0.8 ± 0.1	0.8 - 1.0
Alkaline Phosphatase (IU/ℓ)	22	71.6 ± 5.4	62 - 80
CPK	11	153.0 ± 7.7	139 - 161
LDH	3	98.0 ± 2.4	95 - 101
HBDH	3	226.0 ± 7.2	214 - 238

a/ Performed in December 1976.

TABLE B

REPRODUCIBILITY WITHIN A TEST DAY
ON THE SAME SPECIMEN[a/]

	Mean ± S.D.[b/]	Range
Erythrocytes (x 10^6/mm^3)	5.90 ± 0.14	5.73 - 6.08
Reticulocytes (%)	0.63 ± 0.12	0.44 - 0.79
Hematocrit (vol %)	46.8 ± 0.6	46.0 - 47.5
Hemoglobin (gm %)	16.1 ± 0.2	15.8 - 16.1
Platelets (x 10^5/mm^3)	1.56 ± 0.07	1.49 - 1.66
Leukocytes (x 10^3/mm^3)	10.8 ± 0.4	10.2 - 11.3
Bands (%)	0 ± 0	0 - 0
Neutrophils (%)	64.3 ± 3.1	61 - 69
Lymphocytes (%)	29.0 ± 4.9	23 - 35
Eosinophils (%)	3.2 ± 0.8	2 - 4
Basophils (%)	0 ± 0	0 - 0
Monocytes (%)	3.4 ± 0.9	3 - 5
Atypical (%)	0 ± 0	0 - 0
Nucleated RBC (%)	0 ± 0	0 - 0
Methemoglobin (gm %)	0 ± 0	0 - 0
Fasting Glucose (mg %)	96.7 ± 3.0	32 - 101
SGOT (IU/ℓ)	23.2 ± 2.8	21 - 28
SGPT (IU/ℓ)	25.3 ± 2.1	24 - 28
Creatinine (mg %)	0.6 ± 0.1	0.5 - 0.6
BUN (mg %)	9.0 ± 0.0	9 - 9
Alkaline Phosphatase (IU/ℓ)	63.5 ± 1.1	62 - 65
CPK	44.0 ± 1.6	43 - 46
LDH	38.5 ± 1.6	37 - 40
HBDH	42.0 ± 1.6	40 - 43

a/ Performed in October 1976.
b/ Six determinations from an adult beagle blood sample.

TABLE C

PROFICIENCY TEST SERVICE (PTS) REPORTS (1975-1976)[a/]

Unknowns	MRI Results	PTS Results	Participating Laboratories (10-90 Percentiles) Median	Mean	Acceptable Performance[b/]
Hemoglobin	13.8 gm %	13.8	13.8	13.8	13.6 - 14.0
	18.1 gm %	17.9	17.9	17.8	17.6 - 18.2
Serum Protein	6.6 mg %	7.1	7.0	7.0	6.7 - 7.3
Fasting Glucose	272.0 mg %	264.5	266.0	263.0	240 - 290
	229.0 mg %	221.4	220.5	222.5	200 - 240
BUN	12.1 mg %	12.0	12.0	12.2	11.0 - 13.0
	38.4 mg %	40.1	40.3	39.2	36.0 - 44.0
Creatinine	1.0 mg %	1.0	1.0	1.0	0.8 - 1.3
	4.3 mg %	4.4	4.5	4.4	3.9 - 4.9
Bilirubin	3.9 mg %	4.16	4.15	4.14	3.5 - 4.6
	1.3 mg %	1.78	1.80	1.77	1.5 - 2.1
Cholesterol	175.0 mg %	161.4	161.0	162.0	145 - 175
	100.0 mg %	109.8	109.4	111.0	98 - 120
Ca	15.7 meq/ℓ	15.4	15.4	15.3	14.1 - 16.4
	9.5 meq/ℓ	9.8	9.8	9.8	9.2 - 10.3
Na	156.0 meq/ℓ	155.8	156.0	155.5	153 - 158
K	7.3 meq/ℓ	7.5	7.5	7.5	7.3 - 7.7
Cl	96.0 meq/ℓ	97.8	93.0	97.5	96 - 101
	78.0 meq/ℓ	79.4	79.0	80.0	77 - 83
Mg	1.0 meq/ℓ	1.1	1.1	1.2	0.9 - 1.4
	1.9 meq/ℓ	2.0	2.0	2.1	1.8 - 2.3

a/ To date, we have received unknowns for phosphorus, uric acid, and serum iron. We do not routinely perform these determinations.

b/ Based on values submitted by participants by 10th of month.

TABLE D

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE RHESUS MONKEYS[a/]

	Male Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	108	3.74 ± 0.50	5.51 ± 0.45	3.75 - 6.61
Reticulocytes (%)	108	3.74 ± 0.50	0.97 ± 0.82	0.07 - 2.41
Hematocrit (vol %)	108	3.74 ± 0.50	43.0 ± 2.6	37.0 - 50.0
Hemoglobin (gm %)	108	3.74 ± 0.50	13.4 ± 0.8	10.8 - 15.4
MCV (μ^3)	108	3.74 ± 0.50	77.8 ± 7.0	69.6 - 117.3
MCHb ($\mu\mu$g)	108	3.74 ± 0.50	24.4 ± 1.8	21.0 - 33.6
MCHbC (mg %)	108	3.74 ± 0.50	31.4 ± 1.3	27.2 - 34.1
Platelets (x 10^5/mm^3)	99	3.74 ± 0.50	3.08 ± 0.45	0.80 - 7.10
Leukocytes (x 10^3/mm^3)	108	3.74 ± 0.50	10.4 ± 4.9	3.8 - 30.1
Neutrophils I (%)	108	3.74 ± 0.50	0.18 ± 0.45	0 - 2
Neutrophils M (%)	108	3.74 ± 0.50	39.10 ± 17.72	10 - 83
Lymphocytes (%)	108	3.74 ± 0.50	56.83 ± 17.74	13 - 84
Eosinophils (%)	108	3.74 ± 0.50	1.91 ± 2.42	0 - 13
Monophils (%)	108	3.74 ± 0.50	1.37 ± 1.58	0 - 7
Basophils (%)	108	3.74 ± 0.50	0.04 ± 0.20	0 - 2
Atypical cells (%)	108	3.74 ± 0.50	0.00 ± 0.00	0 - 0
Nucleated RBC (%)		3.74 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	100	3.76 ± 0.51	96.9 ± 15.2	59 - 127
SGOT (IU/ℓ)	100	3.76 ± 0.51	33.7 ± 9.2	20 - 60
SGPT (IU/ℓ)	100	3.76 ± 0.51	31.3 ± 7.8	15 - 46
Alkaline Phosphatase (IU/ℓ)	100	3.76 ± 0.51	360.0 ± 116.0	143 - 501
BUN (mg %)	100	3.76 ± 0.51	19.5 ± 7.5	12 - 65
Proth. Time (sec)	62	3.91 ± 0.44	10.2 ± 0.7	9.3 - 11.9
Serum Creat. (mg %)	100	3.76 ± 0.51	1.1 ± 0.3	0.6 - 1.8
Bilirubin				
Total (mg %)	62	3.91 ± 0.44	0.1 ± 0.2	0.0 - 0.8
Direct (mg %)	62	3.91 ± 0.44	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	62	3.91 ± 0.44	18.0 ± 7.4	2 - 34
Na (mEq/ℓ)	62	3.91 ± 0.44	154.0 ± 19.1	144 - 179
K (mEq/ℓ)	62	3.91 ± 0.44	4.8 ± 0.6	3.9 - 5.7
Cl (mEq/ℓ)	62	3.91 ± 0.44	109.0 ± 6.4	93 - 118
Ca (mEq/ℓ)	62	3.91 ± 0.44	5.2 ± 0.4	4.2 - 6.3
Mg (mEq/ℓ)	62	3.91 ± 0.44	1.6 ± 0.1	1.2 - 1.8
Bone Marrow				
Myeloid/erythroid ratio	15	3.65 ± 0.41	1.5 ± 0.3	1.5 - 2.1

a/ Data collected between June 1971 and December 1976.

TABLE F

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE RHESUS MONKEYS[a/]

	Female Rhesus Monkeys			
	Number Studied	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	81	3.51 ± 0.48	5.33 ± 0.40	4.25 - 6.03
Reticulocytes (%)	81	3.51 ± 0.48	1.07 ± 0.54	0.35 - 3.31
Hematocrit (vol %)	81	3.51 ± 0.48	41.5 ± 2.8	30.0 - 46.0
Hemoglobin (gm %)	81	3.51 ± 0.48	13.1 ± 1.0	7.9 - 14.1
MCV (μ^3)	81	3.51 ± 0.48	77.7 ± 5.3	66.5 - 95.2
MCHb ($\mu\mu$g)	81	3.51 ± 0.48	24.6 ± 1.7	17.6 - 29.7
MCHbC (mg %)	81	3.51 ± 0.48	31.6 ± 1.4	26.6 - 34.2
Platelets (x 10^5/mm^3)	81	3.51 ± 0.48	3.11 ± 1.23	1.85 - 7.90
Leukocytes (x 10^3/mm^3)	81	3.51 ± 0.48	9.5 ± 3.9	3.2 - 24.8
Neutrophils I (%)	81	3.51 ± 0.48	0.10 ± 0.43	0 - 3
Neutrophils M (%)	81	3.51 ± 0.48	36.41 ± 13.32	13 - 56
Lymphocytes (%)	81	3.51 ± 0.48	60.38 ± 13.26	41 - 79
Eosinophils (%)	81	3.51 ± 0.48	2.28 ± 3.10	0 - 18
Monophils (%)	81	3.51 ± 0.48	0.75 ± 0.98	0 - 4
Basophils (%)	81	3.51 ± 0.48	0.05 ± 0.22	0 - 1
Atypical cells (%)	81	3.51 ± 0.48	0.00 ± 0.00	0 - 0
Nucleated RBC (%)	74	3.56 ± 0.50	0.00 ± 0.00	0 - 0
Fasting Glucose (mg %)	81	3.51 ± 0.48	92.1 ± 15.3	57 - 116
SGOT (IU/ℓ)	81	3.51 ± 0.48	32.1 ± 7.6	20 - 70
SGPT (IU/ℓ)	81	3.51 ± 0.48	30.1 ± 7.6	12 - 39
Alkaline Phosphatase (IU/ℓ)	81	3.51 ± 0.48	349.9 ± 112.3	148 - 572
BUN (mg %)	81	3.51 ± 0.48	17.3 ± 4.2	13 - 29
Proth. Time (sec)	59	3.56 ± 0.43	10.5 ± 0.9	9.7 - 12.3
Serum Creat. (mg %)	81	3.51 ± 0.48	1.1 ± 0.3	0.6 - 1.7
Bilirubin				
Total (mg %)	81	3.51 ± 0.48	0.1 ± 0.1	0.0 - 0.8
Direct (mg %)	81	3.51 ± 0.48	0.0 ± 0.0	0.0 - 0.0
BSP 15 min (% ret.)	59	3.56 ± 0.43	16.4 ± 8.3	5 - 34
Na (mEq/ℓ)	59	3.56 ± 0.43	158.2 ± 6.5	147 - 174
K (mEq/ℓ)	59	3.56 ± 0.43	4.8 ± 0.7	3.9 - 6.2
Cl (mEq/ℓ)	59	3.56 ± 0.43	109.0 ± 6.1	95 - 113
Ca (mEq/ℓ)	59	3.56 ± 0.43	5.3 ± 0.5	4.3 - 6.3
Mg (mEq/ℓ)	59	3.56 ± 0.43	1.6 ± 0.2	1.3 - 2.0
Bone Marrow				
Myeloid/erythroid ratio	11	3.49 ± 0.62	1.4 ± 0.3	1.0 - 1.8

a/ Data collected between June 1971 and December 1976.

TABLE F

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	82 ± 17	64 - 122
Spleen (gm)	4.6 ± 1.8	2.0 - 9.3
Kidneys (gm)	15.1 ± 3.8	8.0 - 22.0
Adrenals (gm)	0.73 ± 0.15	0.45 - 0.86
Thyroids (gm)	0.57 ± 1.30	0.37 - 0.81
Testes (gm)	1.29 ± 0.67	0.53 - 3.30
	Relative (per kg body weight)	
	Mean ± S.D.	Range
Liver (gm)	23.4 ± 2.5	18.8 - 30.4
Spleen (gm)	1.25 ± 0.47	0.57 - 2.38
Kidneys (gm)	4.13 ± 0.92	2.20 - 6.43
Adrenals (mg)	201 ± 44	129 - 254
Thyroids (mg)	154 ± 42	86 - 250
Testes (gm)	0.34 ± 0.11	0.18 - 0.53

a/ Data collected between September 1971 and December 1976 from 17 monkeys weighing 3.71 ± 0.48 kg, used as control animals.

TABLE G

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE RHESUS MONKEYS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	83 ± 17	64 - 122
Spleen (gm)	3.8 ± 1.4	2.0 - 6.0
Kidneys (gm)	14.5 ± 2.8	11.0 - 20.0
Adrenals (gm)	0.68 ± 0.16	0.53 - 1.14
Thyroids (gm)	0.60 ± 0.20	0.37 - 1.11
Ovaries (gm)	0.28 ± 0.10	0.14 - 0.45
	Relative (per kg body weight)	
	Mean ± S.D.	**Range**
Liver (gm)	25.4 ± 5.8	19.2 - 37.4
Spleen (gm)	1.16 ± 0.49	0.60 - 1.89
Kidneys (gm)	4.40 ± 0.86	3.20 - 6.25
Adrenals (mg)	212 ± 80	138 - 438
Thyroids (mg)	173 ± 66	97 - 346
Ovaries (mg)	82 ± 28	43 - 140

a/ Data collected between September 1971 and December 1976 from 11 monkeys weighing 3.39 ± 0.58 kg, used as controls.

TABLE H

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE BEAGLE DOGS[a/]

	Male Beagle Dogs				
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Observed Results Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	276	4 - 7	8.3 ± 1.7	5.55 ± 0.73	3.62 - 7.60
Reticulocytes (%)	284	4 - 7	8.3 ± 1.7	0.72 ± 0.46	0.04 - 4.35
Hematocrit (vol %)	276	4 - 7	8.3 ± 1.7	41.6 ± 3.5	31 - 50
Hemoglobin (gm %)	276	4 - 7	8.3 ± 1.7	13.5 ± 1.4	10.0 - 16.9
MCV (μ^3)	276	4 - 7	8.3 ± 1.7	75.6 ± 8.3	56.7 - 127.1
MCHb ($\mu\mu$g)	276	4 - 7	8.3 ± 1.7	24.6 ± 3.0	17.1 - 41.7
MCHbC (mg %)	276	4 - 7	8.3 ± 1.7	32.5 ± 1.5	28.1 - 40.3
Platelets (x 10^5/mm^3)	270	4 - 7	8.4 ± 1.7	2.91 ± 1.02	0.93 - 6.35
Leukocytes (x 10^3/mm^3)	284	4 - 7	8.3 ± 1.7	11.9 ± 3.5	4.6 - 24.6
Neutrophils I (%)	284	4 - 7	8.3 ± 1.7	0.55 ± 1.06	0 - 6
Neutrophils M (%)	284	4 - 7	8.3 ± 1.7	56.81 ± 9.47	22 - 80
Lymphocytes (%)	284	4 - 7	8.3 ± 1.7	37.94 ± 9.26	13 - 71
Eosinophils (%)	284	4 - 7	8.3 ± 1.7	2.76 ± 2.93	0 - 16
Monophils (%)	284	4 - 7	8.3 ± 1.7	1.78 ± 1.84	0 - 11
Basophils (%)	284	4 - 7	8.3 ± 1.7	0.01 ± 0.10	0 - 2
Atypical cells (%)	284	4 - 7	8.3 ± 1.7	0.11 ± 0.37	0 - 2
Nucleated RBC (%)	284	4 - 7	8.3 ± 1.7	0.02 ± 0.10	0 - 2
Fasting Glucose (mg %)	284	4 - 7	8.3 ± 1.7	100.9 ± 12.6	66 - 134
SGOT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	23.2 ± 7.4	11 - 59
SGPT (IU/ℓ)	276	4 - 7	8.3 ± 1.7	25.7 ± 7.9	8 - 46
Alkaline Phosphatase (IU/ℓ)	276	4 - 7	8.3 ± 1.7	73.3 ± 18.5	21 - 133
BUN (mg %)	284	4 - 7	8.3 ± 1.7	12.1 ± 3.3	4 - 23
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	9.4 ± 1.6	1.6 ± 0.4	1.1 - 3.0

a/ Data collected between September 1971 and December 1976.

TABLE I

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF FEMALE BEAGLE DOGS[a/]

	Female Beagle Dogs			Observed Results	
	Number Studied	Age (months)	Body Weight (kg) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	257	4 - 7	6.9 ± 1.3	5.59 ± 0.73	3.27 - 7.75
Reticulocytes (%)	265	4 - 7	6.9 ± 1.3	0.74 ± 0.52	0.04 - 5.05
Hematocrit (vol %)	257	4 - 7	6.9 ± 1.3	42.3 ± 3.5	32 - 51
Hemoglobin (gm %)	257	4 - 7	6.9 ± 1.3	13.7 ± 1.3	11.0 - 18.6
MCV (μ^3)	257	4 - 7	6.9 ± 1.3	76.7 ± 9.7	55.8 - 128.4
MCHb ($\mu\mu$g)	257	4 - 7	6.9 ± 1.3	24.8 ± 3.3	17.1 - 41.6
MCHbC (mg %)	257	4 - 7	6.9 ± 1.3	32.3 ± 1.6	28.7 - 40.4
Platelets (x 10^5/mm^3)	227	4 - 7	6.9 ± 1.3	3.08 ± 1.15	1.08 - 7.95
Leukocytes (x 10^3/mm^3)	265	4 - 7	6.9 ± 1.3	10.9 ± 3.4	3.8 - 26.9
Neutrophils I (%)	265	4 - 7	6.9 ± 1.3	0.54 ± 1.16	0 - 7
Neutrophils M (%)	265	4 - 7	6.9 ± 1.3	57.08 ± 10.10	31 - 85
Lymphocytes (%)	265	4 - 7	6.9 ± 1.3	37.15 ± 10.46	10 - 61
Eosinophils (%)	265	4 - 7	6.9 ± 1.3	2.37 ± 2.25	0 - 13
Monophils (%)	265	4 - 7	6.9 ± 1.3	1.94 ± 2.01	0 - 9
Basophils (%)	265	4 - 7	6.9 ± 1.3	0.01 ± 0.09	0 - 1
Atypical cells (%)	265	4 - 7	6.9 ± 1.3	0.11 ± 0.43	0 - 4
Nucleated RBC (%)	265	4 - 7	6.9 ± 1.3	0.03 ± 0.17	0 - 2
Fasting Glucose (mg %)	248	4 - 7	6.9 ± 1.3	99.6 ± 14.4	55 - 130
SGOT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	23.5 ± 7.2	6 - 52
SGPT (IU/ℓ)	257	4 - 7	6.9 ± 1.3	25.3 ± 7.0	8 - 49
Alkaline Phosphatase (IU/ℓ)	257	4 - 7	6.9 ± 1.3	73.5 ± 19.2	30 - 146
BUN (mg %)	265	4 - 7	6.9 ± 1.3	12.4 ± 3.3	4 - 26
Bone Marrow					
Myeloid/erythroid ratio	34	5 - 9	7.8 ± 1.4	1.4 ± 0.3	1.1 - 2.4

a/ Data collected between September 1971 and December 1976.

TABLE J

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	264 ± 51	166 - 384
Spleen (gm)	58 ± 25	22 - 167
Kidneys (gm)	53 ± 10	32 - 71
Adrenals (gm)	1.12 ± 0.26	0.74 - 1.75
Thyroids (gm)	1.03 ± 0.32	0.55 - 2.50
Testes (gm)	6.60 ± 4.56	1.32 - 18.00
	Relative (per kg body weight)	
	Mean ± S.D.	Range
Liver (gm)	27.9 ± 4.2	19.6 - 42.3
Spleen (gm)	6.0 ± 2.0	2.8 - 12.5
Kidneys (gm)	5.6 ± 0.8	4.0 - 7.7
Adrenals (mg)	117 ± 25	70 - 165
Thyroids (mg)	108 ± 34	56 - 211
Testes (gm)	0.67 ± 0.39	0.13 - 1.67

a/ Data collected between September 1971 and December 1976 from 51 dogs, weighing 9.3 ± 1.8 kg, used as control animals.

TABLE K

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF FEMALE BEAGLE DOGS[a/]

Organ Weight	Absolute Mean ± S.D.	Absolute Range
Liver (gm)	218 ± 51	106 - 322
Spleen (gm)	48 ± 21	16 - 103
Kidneys (gm)	43 ± 9	24 - 71
Adrenals (gm)	1.04 ± 0.26	0.49 - 1.65
Thyroids (gm)	0.88 ± 0.25	0.55 - 1.91
Ovaries (gm)	0.74 ± 0.24	0.38 - 1.27
	Relative (per kg body weight)	
	Mean ± S.D.	Range
Liver (gm)	28.2 ± 5.0	20.7 - 38.8
Spleen (gm)	6.0 ± 2.3	3.1 - 10.9
Kidneys (gm)	5.5 ± 0.9	3.7 - 7.9
Adrenals (mg)	135 ± 35	67 - 215
Thyroids (mg)	112 ± 31	75 - 219
Ovaries (mg)	96 ± 33	54 - 222

a/ Data collected between September 1971 and December 1976 from 49 dogs, weighing 7.7 ± 1.5 kg, used as control animals.

TABLE L

HEMATOLOGY, CLINICAL BLOOD CHEMISTRY VALUES, AND BONE MARROW (MYELOID/ERYTHROID) RATIOS OF MALE ALBINO RATS[a/]

	Male Rats			Observed Results	
	Number Studied	Age (weeks)	Body Weight (gm) Mean ± S.D.	Mean ± S.D.	Range
Erythrocytes (x 10^6/mm^3)	527	5 - 7	168 ± 22	5.84 ± 0.54	3.24 - 7.60
Reticulocytes (%)	461	5 - 7		3.04 ± 1.80	0.30 - 6.83
Hematocrit (vol %)	525	5 - 7	168 ± 22	45.1 ± 3.2	40 - 58
Hemoglobin (gm %)	525	5 - 7	168 ± 22	13.7 ± 0.9	11.8 - 17.1
MCV (μ^3)	525	5 - 7	168 ± 22	78.1 ± 16.3	62.3 - 104.6
MCHb ($\mu\mu$g)	525	5 - 7	168 ± 22	23.7 ± 2.6	19.2 - 41.0
MCHbC (mg %)	525	5 - 7	168 ± 22	30.5 ± 1.8	21.1 - 36.9
Platelets (x 10^5/mm^3)	473	5 - 7	164 ± 24	4.93 ± 1.23	2.30 - 7.95
Leukocytes (x 10^3/mm^3)	448	5 - 7	164 ± 24	15.4 ± 4.0	6.3 - 20.8
Neutrophils I (%)	448	5 - 7	164 ± 24	0.07 ± 0.31	0 - 3
Neutrophils M (%)	448	5 - 7	164 ± 24	14.1 ± 6.2	4 - 29
Lymphocytes (%)	448	5 - 7	164 ± 24	83.63 ± 6.75	52 - 96
Eosinophils (%)	448	5 - 7	164 ± 24	0.64 ± 0.91	0 - 6
Monophils (%)	448	5 - 7	164 ± 24	1.23 ± 1.73	0 - 13
Basophils (%)	448	5 - 7	164 ± 24	0.01 ± 0.15	0 - 2
Atypical cells (%)	448	5 - 7	164 ± 24	0.01 ± 0.12	0 - 2
Nucleated RBC (%)	448	5 - 7	164 ± 24	0.10 ± 0.42	0 - 4
Fasting Glucose (mg %)	125	10 - 12	348 ± 72	130.9 ± 17.2	94 - 165
SGOT (IU/ℓ)	125	10 - 12	348 ± 72	108.2 ± 34.5	63 - 223
SGPT (IU/ℓ)	125	10 - 12	348 ± 72	34.2 ± 16.5	17 - 120
Alkaline Phosphatase (IU/ℓ)	125	10 - 12	348 ± 72	94.9 ± 30.0	32 - 153
BUN (mg %)	125	10 - 12	348 ± 72	16.4 ± 4.7	8 - 41
Bone Marrow					
Myeloid/erythroid ratio	109	10 - 12	349 ± 63	1.7 ± 0.5	1.0 - 2.6

a/ Data collected between September 1971 and December 1976.

TABLE M

ABSOLUTE AND RELATIVE ORGAN WEIGHTS OF MALE ALBINO RATS[a/]

Organ Weight	Absolute Mean ± S.D.	Range
Liver (gm)	10.89 ± 2.87	7.18 - 15.09
Spleen (gm)	0.65 ± 0.11	0.34 - 0.89
Kidneys (gm)	2.64 ± 0.37	1.84 - 3.58
Adrenals (mg)	63.6 ± 9.5	21.9 - 73.5
Thyroids (mg)	26.3 ± 5.8	14.3 - 37.7
Testes (gm)	2.98 ± 0.51	1.76 - 3.81
	Relative (per 100 gm body weight) Mean ± S.D.	Range
Liver (gm)	2.96 ± 0.42	2.09 - 4.01
Spleen (gm)	0.19 ± 0.08	0.10 - 0.30
Kidneys (gm)	0.76 ± 0.10	0.22 - 0.88
Adrenals (mg)	18.6 ± 5.8	5.8 - 22.4
Thyroids (mg)	7.6 ± 2.7	4.2 - 12.7
Testes (gm)	0.87 ± 0.15	0.23 - 1.09

a/ Data collected between September 1971 and December 1976 from 139 rats, weighing 352 ± 59 gm, used as control animals.

TABLE N

PRESENCE OF VARIOUS SUBSTANCES IN THE URINE OF MALE AND FEMALE MONKEYS, DOGS AND MALE RATS

Species:	Monkeys		Dogs		Rats[a]	
No. of Animals:	141[b]	18	615[b]	112	84[b]	18
No. of Collections:	141	98[c]	615	565[c]	84	56[d]
Glucose: < 250 mg %	0[e]	2.0 (2)	0.2 (1)	0.7 (4)	0	0
> 250 mg %	0	0	0.5 (3)	0.2 (1)	0	0
Protein: < 100 mg %	3.5 (5)	6.1 (6)	19.3 (119)	17.3 (98)	29.8 (25)	36.0 (18)
> 100 mg %	0	2.0 (2)	2.3 (14)	1.8 (10)	0	0
RBC:[f] Moderate	1.4 (2)	3.1 (3)	16.4 (101)	13.3 (75)	3.6 (3)	8.0 (4)
Excessive	0	0	3.4 (21)	3.2 (18)	0	0
WBC:[f] Moderate	1.4 (2)	2.0 (2)	18.7 (115)	20.9 (118)	0	4.0 (2)
Excessive	0	0	3.9 (24)	3.7 (21)	0	0
Epithelium:[g] Moderate	31.2 (44)	44.9 (44)	21.0 (129)	21.9 (124)	0	8.0 (4)
Excessive	3.5 (5)	7.1 (7)	4.7 (29)	2.8 (16)	0	0
Crystal:[h] Moderate	0.7 (1)	2.0 (2)	0.2 (1)	0.7 (4)	0	2.0 (1)
Excessive	0	0	0.2 (1)	0.7 (4)	0	2.0 (1)
Casts: Positive	0.7 (1)	5.1 (5)	0	0.9 (5)	0	0

a/ Pooled sample of 4-20 rats.
b/ Baseline data collected from all animals employed between September 1971 and December 1976.
c/ Data collected at weekly intervals for 4-7 collections from controls employed between September 1971 and December 1976.
d/ Data collected at 2-week intervals for 2-4 collections from control rats employed between September 1971 and December 1976.
e/ Percent of total (number of samples).
f/ Normal, 10 or less cells; moderate, 10-100 cells; excessive, > 100 cells/field (x 440).
g/ Normal, 5 or less cells; moderate, 5-25 cells; excessive, > 25 cells/field (x 100).
h/ Normal, none; moderate, 1-5 crystals; excessive, > 5 crystals/field (x 100).

TABLE 0

PRESENCE OF OCCULT BLOOD IN THE FECES OF MALE AND FEMALE MONKEYS AND DOGS

Species:		Monkeys		Dogs	
No. of Animals:		44[a]	8	118[a]	30
No. of Collections:		44	48[b]	118	156[b]
Occult Blood:	Negative	90.9 (40)[c]	95.8 (46)	94.1 (111)	91.7 (143)
	Positive	9.1 (4)	4.2 (2)	5.9 (7)	8.3 (13)

a/ Baseline data collected from all animals employed between July 1974 and December 1976.

b/ Data collected at weekly intervals for 4-7 collections from controls employed between July 1974 and December 1976.

c/ Percent of total (number of samples).

APPENDIX II

MANUAL FOR

STUDY OF DEVELOPMENTAL TOXICITY

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STUDY OF DEVELOPMENTAL TOXICITY

I. INTRODUCTION

The thalidomide catastrophe provides an unfortunate example of the need for reliable information concerning the effects of agents on human development. Prospective and retrospective epidemiological studies are the only ethical procedures currently available to obtain this information in humans. There are, however, a variety of protocols available to obtain preliminary developmental toxicity information in animals. This preliminiary animal information can be used to form the basis from which it is possible to evaluate the risk of exposing the human population to potentially toxic agents.

The purpose of this manual is to describe the protocol used in our laboratory to obtain developmental toxicity information. Sections, in addition, are included which discuss both the statistical analysis and interpretation of the data. A working definition of common anomalies is presented. These studies are based on "The Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use" distributed by the U.S. Food and Drug Administration, 1966, and "The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity" distributed by the Ministry of Health and Welfare, Canada, 1973.

II. PROTOCOL FOR STUDY OF DEVELOPMENTAL TOXICITY

A. Fertility and General Reproductive Performance Study

1. Objectives

The emphasis in this phase is placed on determining the effect of an agent on gonadal function, estrus cycle, mating behavior, conception rates, and the early stages of development. This study serves as an overall pilot screening of the agent on the entire reproductive process including organogenesis, late stages of gestation, parturition, and lactation. The results obtained from this phase serve as a guide for conducting subsequent studies in greater depth.

2. Method

The rat is the animal generally used for this study and both males and females are used to provide an adequate study of fertility. Male rats,

at least 40 days of age, are treated for 60 to 80 days prior to mating to determine if the agent affects spermatogenesis. Male animals from subacute or chronic toxicity studies may be used and each male, from a group of at least 10 animals, is bred with two non-treated females. Each male is exposed overnight to females in proestrus or early estrus until (1) a male mates with two females or (2) a male is exposed, on at least three different occasions, to a total of at least five receptive females. A female is considered receptive if there is an estrous vaginal smear the morning following exposure. This procedure minimizes attributing male infertility to sexual inexperience.

Sexually mature female rats are treated for at least 14 days prior to mating with untreated males. The stages of the estrous cycle are determined by vaginal smears to verify that the animals cycle normally and to detect possible treatment effects on the duration of the estrous cycle. The occurrence of copulation is established by daily vaginal inspection for the presence of sperm. The day on which evidence of copulation is discovered is identified as being day 0 of gestation. Confirmation of pregnancy, however, is not obtained until the animal is sacrificed on day 13 of gestation or delivers a litter at the end of gestation. Females treated prior to mating are continued on the same treatment schedule until the time of sacrifice.

Half of the females from each group are sacrificed on day 13 of gestation. The dams are examined for number of corpora lutea and implantation sites, number and distribution of embryos in each uterine horn, presence of empty implantation sites, embryos undergoing resorption, and any abnormal conditions. The following parameters are determined:

a. Number of viable litters (litters with one or more viable implants)
b. Corpora lutea/dam
c. Total implants/dam
d. Viable implants/dam
e. Indexes of
   (1) Fertility: confirmed pregnancies/sperm positive females
   (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
   (3) Implantation: implants/corpora lutea
   (4) Implant viability: viable fetuses/implants

The remaining dams are allowed to deliver and the litters are examined at birth, day 4, and day 21. The litters are examined for number, weight, mortality, and abnormalities of the pups. The following parameters are determined:

a. Number of viable litters (litters with one or more viable pups)
b. Pups/dam
c. Weight of pups
d. Indexes of
   (1) Fertility: confirmed pregnancies/sperm positive females
   (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
   (3) Implant viability: viable pups/implants
   (4) Viability: pups alive at day 4/pups alive at birth
   (5) Lactation: pups alive at day 21/pups alive at day 4

B. Teratology Study

1. Objectives

The objective of this phase is to determine if an agent has a potential for producing embryotoxicity and/or teratogenicity. Treatment, therefore, is restricted to the period of organogenesis. Dosage may be high during this brief treatment period in order to obtain results concerning teratogenic potential and risk.

2. Method

Two species of animals are employed in this test. The species most frequently used are the mouse, rat, and rabbit. Drug treatment covers the period of organogenesis which is day 6 through 15 of gestation for the mouse and rat and day 6 through 18 for the rabbit.

Sexually mature virgin mice are obtained from reputable suppliers and conditioned in our animal quarters for 10 days. The conditioning period permits the animals to stabilize and establish regular estrus cycles of 4 to 5 days in duration. Females are placed overnight with a non-treated proven male breeder and examined the next morning for evidence of copulation. Successful mating is identified by the presence of a vaginal copulatory plug. The day that plugs are discovered is identified as day 0 of gestation. Mice are sacrificed on day 18 of gestation for fetal examination.

Sexually mature virgin female rats are obtained and conditioned as previously described for mice. Females are examined by vaginal lavage late in the afternoon for signs of proestrus (75-90% of nucleated epithlial cells). Females in proestrus are placed overnight with an experienced male. The following morning, females are examined for sperm or the presence of a vaginal

plug. The plug, however, is not as reliable an indicator of successful mating in rats as it is in mice. Rats are sacrificed on day 20 of gestation and examined for fetal anomalies.

Virgin female rabbits, 6 to 8 months of age, are obtained from commercial sources and are conditioned for 18 days in our animal quarters. Ovulation is induced by the intravenous administration of 1 mg/kg pituitary lutenizing hormone. Females are artificially inseminated within 1 hour by the method of Gibson, et al.[1] Fetuses are delivered by cesarean section on days 27 to 28 of pregnancy and examined for anomalies.

Mouse, rat and rabbit dams are sacrificed by CO_2 anesthesia prior to delivery since many animals tend to cannabilize their defective offspring. A laparotomy is performed and the uterine horns are exposed. The number of corpora lutea and number and position of live, dead, and resorbed fetuses is recorded. The umbilical cord is clamped and severed distally in order to prevent blood loss. Fetuses are removed, weighed and immediately examined by experienced personel for external anomalies as fully described by Wilson.[2]

One-half of the rodent fetuses from each litter are dissected and examined for soft tissue anomalies by the free-hand slicing method of Wilson.[2] Each fetus is fixed in 20 to 25 ml of Bouins fluid for 2 weeks. The hardened fetuses are examined for external anomalies and serially cut from the head through the trunk into 1 mm thick sections using a sharp razor blade. No slices are made beyond the kidneys and the intestines are carefully removed from the pelvic cavity. The cross sections of the fetuses and the genitourinary organs on the pelvic floor are carefully examined by experienced personnel. The remaining fetuses from each litter are processed for skeletal examination. Fetuses are fixed in 70% alcohol for 2 weeks and eviscerated. The fetuses are stored in 1% KOH for 2 days and then stained with alizarin red.[3] After differential decolorization, the skeletons are examined by experienced personnel for anomalies. For rabbits, all fetuses are examined for both soft tissue and skeletal defects.

C. Perinatal and Postnatal Study

1. Objectives

The purpose of this phase of the protocol is to determine the effect of drugs administered during the last third of pregnancy and the period of lactation. The specific areas of study are the drug effects on late fetal development, labor and delivery, lactation, neonatal viability, and growth of the newborn.

2. Method

The conditioning, mating, and establishment of pregnancy in rats and mice are as previously described. The drug is administered to the dam during the final one-third of gestation and continued throughout lactation to weaning. The test compound is incorporated into the diet and a pair-fed control group, whose food intake is limited to the least amount of food consumed by the treated group, is included in the study. Treatment in rats and mice is initiated on day 16 of gestation and continued until the pups are weaned at 21 days of age. Labor and delivery are observed whenever possible and any signs of abnormal, prolonged, or delayed labor are carefully noted. The duration of gestation is calculated for each mother in all groups. The litters are examined as soon as possible after delivery, and at 4 and 21 days of age. The examination of the pups is conducted with a minimum disturbance of the mother. The following information is recorded for all the litters in each group:

a. Litter size
b. Number of stillborn and live born
c. Anomalies of dead and live pups
d. Number and weight of pups at 4 and 21 days of age
e. Indexes of
   (1) Fertility: confirmed pregnancies/sperm positive females
   (2) Gestation: confirmed pregnancies with viable fetuses/confirmed pregnancies
   (3) Viability: pups alive at day 4/pups alive at birth
   (4) Lactation: pups alive at day 21/pup alive at day 4

III. STATISTICAL ANALYSIS OF DATA

Two important considerations in performing a valid statistical analysis are the determination of the sample size and the selection of appropriate statistical tests. In a study of developmental toxicity the sample size is determined by the selection of experimental units. The litter, rather than individual fetuses, is considered to be the unit of observation for our studies since the dam is the unit of treatment and the fetal response is dependent, to some degree, on maternal influences.

The data collected fall into two categories. The first category is enumeration or discontinuous data. Examples of discontinuous data are the number of sperm positive animals with evidence of conception, mortality, and indexes of fertility and gestation. The Fisher Exact Probability Test[4/]

is the test of choice to evaluate the significance. Such enumeration data are reported with the exact 95% confidence limits.

The second category is quantitative or continuous data. Examples of continuous data are body weight, food consumption, and the remaining indexes. Such quantitative data are reported as the mean ± the standard error (S.E.). These data are analyzed by Bartlett's test[5] for homogeneity. The tests of significance for homogeneous data are either Dunnett's procedure (one control group) or Tukey's omega procedure (more than one control group). If heterogeneity is indicated, then significance is based on multiple comparisons with the nonparametric rank test.[6] The level of significance is selected as $P < 0.05$.

IV. INTERPRETATION OF DATA

A. Phases of Fetal Development

The development of an adult organism from a single cell may be divided into six phases.[7] The first phase of development, gametogenesis, involves the growth and maturation of the egg and sperm. The gametes fuse during the second phase of development and the quiescent egg is activated to continue its developmental program. Cleavage, the third phase of development, encompasses a period of rapid cell division without a significant change in embryonic size or cellular differentiation. The embryo, at the end of cleavage, is referred to as a blastula and consists of a layer of cells, the blastoderm, surrounding a cavity, the blastocoele. The embryo attaches to the uterine wall and begins the process of placentation at the blastula stage. Gastrulation is the fourth developmental phase and involves the formation of germinal layers from the blastoderm. Primary organ rudiments are derived from the germinal layers during organogenesis, the fifth phase of development. The sixth phase of development is a period of growth and histological differentiation. The organ rudiments grow during this period and acquire the structure and biochemical properties characteristic of adult tissues. Organs grow by increasing both the number and size of cells. Tissue specific characteristics are established by a differential expression of the genetic information.

Treatments may affect the various phases of both animal and human development. The protocol used in our laboratory is designed to determine the developmental toxicity of a treatment in laboratory animals. The various parameters used to measure developmental toxicity help to determine if an agent affects any of the six developmental phases previously described. Since it is not practical to study each phase of development separately, the various phases are combined into periods of study. The units of study are the pre-implantation period (phases 1-3), post-implantation period (phases 3-5), and the period of differentiation (phase 6).

The dam and developing animal represent an integrated unit during the time of treatment. Effects which are observed in the developing animal, therefore, may be due to toxicity of the treatment in either the dam or developing animal. As development progresses it becomes more difficult to attribute an effect to a single period of study or treatment.

B. Fertility and General Reproductive Performance Study

The fertility and general reproductive performance study involves treating females during all six phases of development and treating males only during the period of gametogenesis. The effect of the treatment in females is studied at mid-gestation and after delivery. Males, on the other hand, are mated with normal females and treatment effects are studied in these females at mid-gestation and after delivery.

Some females are examined at mid-gestation and the various parameters previously described are recorded. The number of corpora lutea are counted by gross inspection and this value provides a measure of the ova released during ovulation. The number of implantations is used as a measure of the fertilized ova that developed to a stage where an attachment to the uterine wall is obvious at the time of inspection. The observations are summarized in the form of indexes. The fertility index is the percentage of mated females that are pregnant. A reduction in this index reflects pre-implantation losses. The implantation index is the percentage of ova that implant and it also provides a measure of pre-implantation losses. The implant viability index is the percentage of implants which appear normal at the time of examination. A reduction in this index serves as an indication of post-implantation losses. The gestation index is the percentage of pregnant females with one or more viable embryos and provides a measure of post-implantation survival.

Some females are examined after birth and the growth and development of the pups is recorded as previously described. Effects observed at this time may have been produced at any of the six developmental phases. The observations are summarized in the form of indexes. The fertility index provides a measure of pre-implantation losses. The gestation and implant viability indexes calculated on the basis of pups rather than embryos, provide an indication of post-implantation losses. The viability index is the percentage of live-born pups which survive to day 4. A reduction in this index reflects an effect at the post-implantation or differentation period since normal pups can survive for brief periods without maternal care. The lactation index is the percentage of pups alive on day 4 which survive to day 21 and is a measure of effects occuring during the period of treatment. A reduction in this index reflects an impaired ability of the mother to nourish the young, the passage of toxic material to the young through the milk, and/or the manifestation of a developmental defect.

Effects observed at the mid-gestation or postnatal examination in females mated with treated males are indicative of toxicity produced during spermatogenesis, the first phase of development. Abnormalities in sperm may be manifested at any of the developmental stages beginning with fertilization. The previously described parameters are used to identify these effects.

C. Teratology Study

The teratology study involves treating pregnant females during the period of organogenesis and observing fetuses prior to term in order to identify possible effects on development. Treatment of rodents from day 6 through 15 of gestation roughly corresponds to developmental stages 3 to 5 which are in the post-implantation period. If evidence of toxicity is observed during fetal examination, then a primary effect was produced at any of these stages. The primary effect may be compounded into a series of secondary effects as development progresses.

Malformations may fall into three groups.[8/] The first group is common variations and includes retarded ossifications. The second group is minor anomalies and refers to effects such as malformed sternabrae, wavy ribs, and supernumerary ribs. The third group is major malformations and includes anomalies which seriously affect the growth and survival of the offspring. Malformations are not equally significant or useful in interpreting or extrapolating animal experimental studies to man. Anomalies such as supernumerary ribs and decreased or abnormal sternal ossification patterns, for example, might be of little importance both to the animal and to attempts at predicting toxicity in humans. Malformations of doubtful significance include curly tail, straight legs, malrotated limbs and paws, wrist drop, protruding tongue, enlarged atria and/or ventricles, abnormal renal pelvic development and translucent skin.

The defects are reported as an anomaly index. The percent of the fetuses with a given defect is calculated for each litter and these values are then averaged and presented as the mean $\pm$ standard error (S.E.). The mean value provides a measure of the affected fetuses per litter for the group and the standard error provides an estimation of the distribution of the effect between litters within the group.

D. Perinatal and Postnatal Study

This study involves treating the dam during both the later portion of developmental phase 5 and most of phase 6. The growth and development of the pups is observed to monitor possible developmental toxicity. The various

indexes which are used to summarize these observations are discussed above in the section on Interpretation of the Fertility and General Reproductive Performance Study.

V. DISCUSSION OF PROTOCOL

A variety of experimental protocols are available to obtain information concerning the effects of agents on reproduction and development. An aim of these animal studies is to provide information concerning the risk of exposing the human population to chemical agents. The procedure used in our laboratory to obtain this information complies with the FDA guidelines for general reproduction, teratology, and perinatal and postnatal studies. There are problems associated with conducting and evaluating the results.

A. Problems Conducting Protocol

1. Selection of Test Animal

The ideal test animal should (1) absorb, metabolize and eliminate the test substance the same way as humans, (2) transmit the substance and its metabolites to the developing animal at the same rate as humans, and (3) have embryos, fetuses, and neonates with the same development schedules and metabolic pathways as the developing human. The existing comparative data is insufficient to determine which animal species is most like man in any of these characteristics. The currently available information, however, indicates that no presently used species, including simian primates, is like man in all of these respects.[9] The degree of similarity to man that a given species exhibits may vary from one test substance to another. The above criteria for an ideal test animal should be considered, as far as the available information permits, in the selection of test species. The advantages and disadvantages of species commonly used for these tests are:

a. Mouse: The mouse is inexpensive to maintain in large breeding colonies and its embryology is well documented. Small size with a limited supply of tissues and body fluids is a disadvantage in the examination of fetuses for defects and in studies on absorption, metabolism, and excretion of chemical agents. Mice respond to some substances that have limited teratogenicity in other animals and have earned the reputation for unusual sensitivity to teratogens.[9]

b. Rat: The rat has a convenient size for evaluation and analytical purposes, high fecundity, and a low incidence of spontaneous malformations. There is, however, no adequate single source of information on rat embryology, although this information is covered in numerous research papers.

c. Rabbit: The large size of this species permits the collection of large amounts of body fluids and tissues for analysis. Disease and parasites present obstacles to high reproductive performance in some laboratories and good stocks of rabbits are not universally available. The embryology is not fully documented for rabbits but is adequate for most purposes. Since this species was among the first animals to respond teratogenically to thalidomide, rabbits have been credited with greater similarity in teratogenic sensitivity to man than is warranted. There is no reason to regard the rabbit rather than the various species of rodents, which are their close relatives, as a more valid test animal for evaluating the teratogenic risk of agents in humans.[8/]

2. Selection of Dosage

Problems associated with selecting the dosage are the route, amount and duration of treatment. The practice of administering test substances to animals by the same route that will be used clinically is sound. If animals are treated orally for a short period of time, as in teratology studies, then gastric gavage is preferred to incorporating the agent into the diet. A stomach tube permits the accurate administration of a dose and eliminates the variables of food wastage and possible chemical change as a result of exposure to air, light, and other dietary ingredients. Prolonged treatment of animals by gastric gavage is not practical, however, due to the increased risk of trauma and expense associated with daily animal treatments. Agents incorporated into an animal's diet may alter the normal food intake as a result of an effect on appetite or a disagreeable odor or smell. Pair feeding, therefore, is required to determine the effect of reduced feed consumption on growth and development.

The dose levels should include a dose which produces maternal toxicity. The rationale for selecting this dose is to ensure that a maternal response is produced. Maternal toxicity may be measured in terms of lethality, weight loss or any other parameter that is related to treatment. If development is disrupted at doses which produce maternal toxicity, then lower doses should be studied in order to identify a dose below which no effect is observed on development. The identification of a dose which produces neither adult nor developmental toxicity is of value in estimating a safe dose for humans.

Animals are treated throughout various phases of development in this protocol to determine the effect of the agent on development. A treatment schedule which involves prolonged drug exposure presents three basic problems which affect the actual level of drug exposure and the detection of developmental toxicity. First, prolonged drug exposure may increase the activity of the drug metabolizing enzymes which are responsible for the biotransformation of chemicals. The metabolism of the test compound, therefore, is increased; maternal blood levels of the parent compound are decreased;

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and maternal exposure to metabolites may be increased. Second, prolonged drug exposure may produce liver and/or kidney damage. A reduction in the functional capability of the liver reduces the biotransformation of the test compound while impaired kidney function may reduce the elimination of the drug from the body. Third, if a compound is administered during the early portion of gestation, then implantation and early embryonic survival may be impaired. The presence of small litter size and a high degree of resorption prevents the detection of teratogenic effects.

The length of gestation in most experimental animals is short compared to that of humans. Treating experimental animals during gestation may not produce tissue levels which could occur from more prolonged drug exposure as in human pregnancy. This difficulty can, in some cases, be overcome by increasing the dose, but problems may arise if the drug is poorly absorbed or degraded prior to absorption.

3. Determination of Feed Consumption

Animals may be treated during developmental toxicity studies by incorporating the test compound(s) into their diet. The compounds may represent either a fixed or variable percent of the diet. Since feed consumption varies during gestation and lactation, it is advisable to administer the drug as a variable percent of the diet in order to administer a constant amount of drug. The drug intake can be calculated from the percent of the drug in the diet and the amount of feed consumed. Thus, an accurate estimation of feed intake is imperative.

Accurate measurement of feed intake in laboratory animals, especially in rodents, is difficult due to spillage. Feed can be given to rats in stainless steel diet feeders (Model HB-69, Hoeltge, Cincinnati, Ohio) and to mice in stainless steel compartment feeders (Lab Products Inc., Garfield, New Jersey) which are designed to eliminate spillage. In most cases, these feeders are spill-proof; however, animals occasionally acquire the necessary skill to defeat the feeder. When feed consumption is high and the spillage can be measured, then the true feed consumption is calculated. If, on the other hand, the spillage can not be reasonably estimated, the result is omitted.

B. Problems Interpreting the Data

The ultimate goal of testing drugs in animals is to obtain information for making predictive statements concerning a drug's effect in humans. There are problems inherent to animal experiments, particularly in reproduction and teratology studies, which make this extrapolation especially difficult. After the data from a developmental toxicity study have been collected

and analyzed statistically, it is necessary to determine both the significance of defects on normal adult animals and the relevance of the defects to humans.

When developing animals are examined at various times after treatment, evidence of deviant development, as demonstrated by growth retardation, malformations, intrauterine death and functional defects, may be apparent. These observations, however, do not provide information concerning the consequences of these effects in the adult. Growth retardation, for example, may be present in fetuses during a teratology study but may be absent in the adult as a result of maturation and compensatory growth processes. A delayed ossification of bones and the presence of extra ribs are examples of defects which may be corrected during growth or present a problem of questionable significance to the adult. The relevance of these defects to normal growth are, in some cases, difficult to assess experimentally.

The variation between species in response to agents presents the major obstacle to achieving the ultimate goal of any drug testing program. These unique responses of species to agents may be due to metabolic and pharmacokinetic factors.[10] The complexity of the animal system and degree of interspecies variability increases during development as a result of the formation of a placenta and its influence on drug transport, and a changing embryonic sensitivity to drugs. The ability to demonstrate developmental toxicity, therefore, depends on biotransformation of the drug by the mother, placenta, or embryo; pharmacokinetic properties of the drug in the mother and embryo; and embryonic sensitivity at the time of treatment.

VI. TERMINOLOGY OF ANOMALIES

A. Gross Anomalies

1. General

edematous - abnormal accumulation of clear fluid under the skin
hematoma - a localized mass of extravasated blood that is relatively or completely confined within an organ or tissue; not as a result of cesarean section handling
immature skin - skin is sticky with a shiny appearance

2. Head

anophthalmia - absence of one or both eyes
brachygnathia - abnormal shortness or recession of the mandibles
cranium, domed - excessively domed cranium suggestive of hydrocephalus

exencephalus - skull defective, the brain is exposed or extruded
eye, open - eyeball exposed with lids absent or withdrawn
lip, cleft - fissure in the lip, usually causing conjunction of nasal passage and mouth
meningocele - skin intact, translucent, and elevated by a fluid filled vesicle of meninges which protrude through a midline defect in the cranium
meningoencephalocele - meninges and part of brain protruding through a cranial defect to cause an irregular mass beneath the skin
microphthalmia - small or rudimentary eyes
palate, cleft - fissure in hard palate, due to a failure of the palatine shelves to unite
platycephaly - flatness of the skull

3. Trunk

anus, closed - (imperforate anus) anus closed by a membrane so as to prevent the normal passage of intestinal contents
gastroschisis - protrusion of intestines and other abdominal viscera through a ventral midline defect
kyphosis - convexity backward, dorsal-ventral curvature of the spine
myelomeningocele - absence of the vertebral arches through which the spinal cord and its membranes protrude, denoted by a bubble-like bulge along the dorsal midline
rhachischisis - congenital fissure of the spinal column with failure of the skin and vertebral column to close
spina bifida - absence of the vertebral arches through which the spinal membranes, with or without the spinal-cord tissue, protrude, denoted by a raw, usually bloody depression
umbilical hernia - protrusion of intestines through a small ventral midline defect

4. Extremities

acaudate - no tail
adactyly - absence of digits
club foot - abnormal flexion of the foot
micromelia - rudimentary limbs
oligodactyly - fewer than five digits
polydactyly - more than five digits
syndactyly - fused or webbed digits
tail, short - tail is less than half the normal length

REFERENCES

1. Gibson, J.P., R.E. Staples, and J.W. Newberne, "Use of the Rabbit in Teratology Studies," Toxicol. and Appl. Pharmacol., 9: 398-408 (1966).

2. Wilson, J.G., "Methods for Administering Agents and Detecting Malformations in Experimental Animals," in Teratology--Principles and Techniques, J.G. Wilson and J. Workany (eds.), University of Chicago Press, Chicago, Illinois, pp. 262-277 (1965).

3. Staples, R.C. and V.L. Schnell, "Refinements in Rapid Clearing Techniques in the KOH-Alizarin Red S Method for Fetal Bones," Stain Technol., 39: 61-63 (1964).

4. Siegel, S., Nonparametric Statistics, McGraw Hill, New York (1956).

5. Steel, R.G.D. and J.H. Torrie, Principles and Procedures of Statistics, McGraw-Hill Book Co., New York (1960).

6. Mann, H.B., and D.R. Whitney, "On a Test of Whether One of Two Random Variables is Stochastically Larger Than the Other," Ann. Math. Stat., 18: 50-60 (1947).

7. Berrill, N.J., Developmental Biology, McGraw-Hill Book Co., New York (1971).

8. Palmer, A.K., "Spontaneous Malformations of the New Zealand White Rabbit. The Background to Safety Evaluation Tests," Lab. Anim., 2: 195-206 (1968).

9. Wilson, J.G., Environment and Birth Defects, Academic Press, New York, New York (1973).

10. Wilson, J.G., "Factors Determining the Teratogenicity of Drugs," Ann. Rev. Pharmacol., 14: 205-217 (1974).

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